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# Health Assessment Document for Hexachlorocyclopentadiene. Final Report

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# Health Assessment Document for Hexachlorocyclopentadiene

# Final Report

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The Office of Health and Environmental Assessment of the Office of Research and Development has prepared this health assessment document (HAD) at the request of the Office of Air Quality Planning and Standards. Hexachlorocyclopentadiene (HEX) is an intermediate in the pesticide and flame retardant manufacturing processes and is currently being studied by the Environmental Protection Agency (EPA) to determine if it should be regulated as a hazardous air pollutant under the Clean Air Act.

The scientific literature and key studies have been reviewed and evaluated and summaries and conclusions have been directed at the health effects from exposure to HEX. This peer-reviewed document contains information on HEX covering toxicity, environmental fate and transport, aquatic effects, mutagenicity, epidemiology, teratogenicity and other health effects data.

The data base is neither extensive nor adequate for assessing the carcinogenicity or long-term health effects of acute or low-level chronic exposure to HEX.

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# HEALTH ASSESSMENT DOCUMENT
# FOR
# HEXACHLOROCYCLOPENTADIENE

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Cincinnati, Ohio 45268

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# PREFACE

The Office of Health and Environmental Assessment of the Office of Research and Development has prepared this Health Assessment Document (HAD) at the request of the Office of Air Quality Planning and Standards. Hexachlorocyclopentadiene (HEX) is an intermediate in the pesticide manufacturing process and is currently being studied by the Environmental Protection Agency (EPA) to determine if it should be regulated as a hazardous air pollutant under Section 112 of the Clean Air Act.

The scientific literature has been searched and inventoried, key studies have been reviewed and evaluated and summaries and conclusions have been directed at identifying the health effects from exposure to HEX. At several stages in the HAD development process, the HEX document has been reviewed for scientific and technical accuracy. These peer reviews have been by scientists from inside and outside the EPA. Observed effect levels and dose-response relationships are discussed where appropriate in order to identify the critical effect and to place adverse health responses in perspective with observed environmental effects.

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Finis Cavender
Dynamac Corporation
11140 Rockville Pike
Rockville, MD 20852

Shane Que Hee
Department of Environmental Health
University of Cincinnati
Cincinnati, OH

W. Bruce Peirano
Environmental Criteria and Assessment Office
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Randall J.F. Bruins
Environmental Criteria and Assessment Office
Environmental Protection Agency
Cincinnati, OH 45268

Charles H. Nauman
OHEA - Exposure Assessment Group
U.S. Environmental Protection Agency
Washington, DC 20460

Dharm V. Singh
OHEA - Carcinogen Assessment Group
U.S. Environmental Protection Agency
Washington, DC 20460

Sheila Rosenthal
OHEA - Reproductive Effects Assessment Group
Washington, DC 20460

The following individuals provided reviews of this publication and/or earlier drafts of this document:

## U.S. Environmental Protection Agency

### Environmental Criteria and Assessment Office

Michael Dourson
Linda Erdreich
Richard Hertzberg
Franklin Mink
Jennifer Orme
William Pepelko

### Office of Toxic Substances

Ralph Northrop
Carol Glasgow
Harold Day

### Office of Air Quality Planning and Standards

Tim Mohin, OAQPS Project Manager
Larry J. Zaragoza

## CONSULTANTS, REVIEWERS AND CONTRIBUTORS

James R. Withey
Health and Welfare, Canada
Foods Directorate
Ross Avenue
Tunney's Pasture
Ottawa, Ontario
Canada  K1A 0L2

Fumio Matsumura
Pesticide Research Center
Michigan State University
East Lansing, Michigan  48824

Joseph F. Borzelleca
Division of Toxicology
Department of Pharmacology
Medical College of Virginia
Richmond, Virginia  23298

Kamal M. Abdo
NIEHS
P.O. Box 12233
Research Triangle Park, NC  27709

C. Scott Clark
Department of Environmental Health
University of Cincinnati
Cincinnati, Ohio

James G. Colson
Occidental Chemical Corporation
Long Road
Grand Island, New York  14072

Alfred A. Levin
Velsicol Chemical Corporation
341 East Ohio Street
Chicago, Illinois  60611

Jack L. Egle
Medical College of Virginia
Richmond, Virginia  23298

## DOCUMENT PRODUCTION

Technical Support Services Staff: C.A. Cooper, P.A. Daunt, E.R. Durden, C.L. Fessler, K.S. Mann, J.A. Olsen, B.L. Zwayer, Environmental Criteria and Assessment Office, Cincinnati

## Hexachlorocyclopentadiene Peer Review Panel Members

June 29, 1983 Cincinnati, Ohio

Co-chairmen:

Jerry F. Stara, ECAO-CIN
David J. Reisman, ECAO-CIN
Finis Cavender, The Mitre Corporation

### Panel Members

| | |
|---|---|
| James Withey | Health and Welfare, Canada |
| Frederick Coulston | Coulston International |
| Mary Anne Zanetos | Battelle Memorial Institute |
| C. Ralph Buncher | University of Cincinnati |
| Fumio Matsumura | Michigan State University |
| Wyman Dorough | University of Kentucky |
| Joseph Borzelleca | Medical College of Virginia |
| Shane Que Hee | University of Cincinnati |
| Charles H. Nauman | U.S. EPA, OHEA |
| Randall J.F. Bruins | U.S. EPA, ECAO-CIN |
| W. Bruce Peirano | U.S. EPA, ECAO-CIN |
| Linda S. Erdreich | U.S. EPA, ECAO-CIN |
| Richard C. Hertzberg | U.S. EPA, ECAO-CIN |
| Ralph Northrop | U.S. EPA, OTS |
| John Kominsky | U.S. DHHS, NIOSH |
| Alfred A. Levin | Velsicol Chemical Corp. |
| Mildred S. Root | Velsicol Chemical Corp. |
| James Grutsch | Velsicol Chemical Corp. |

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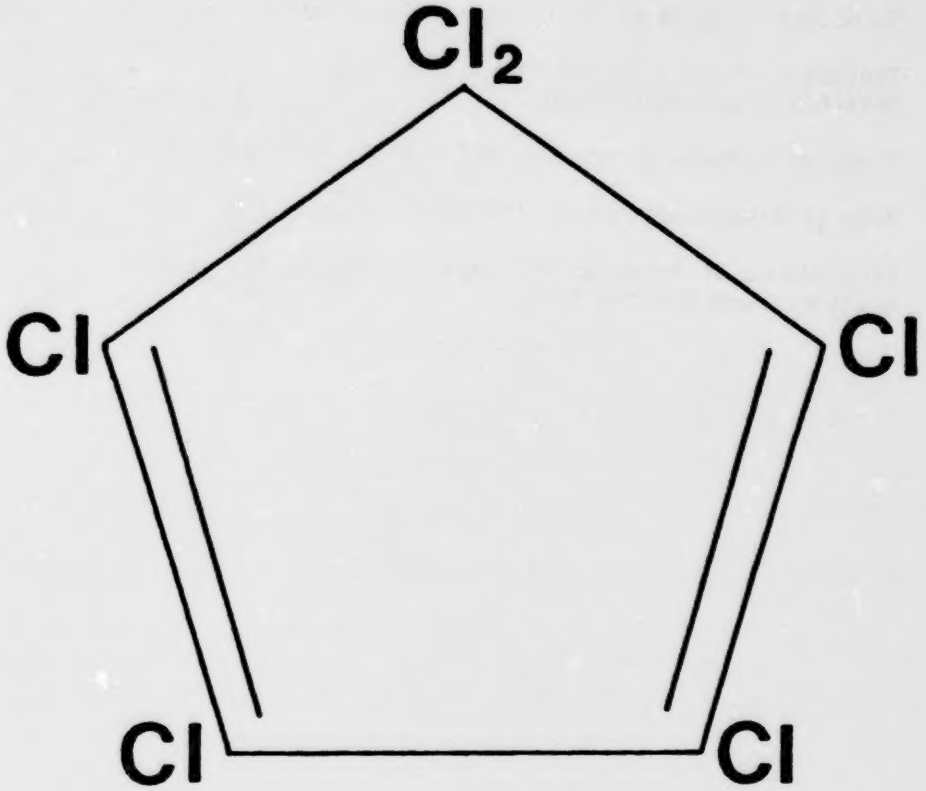


Cl2
Cl
Cl
Cl
Cl

FIGURE 1

Structure Diagram of Hexachlorocyclopentadiene

# 1. INTRODUCTION

Hexachlorocyclopentadiene (HEX) is an unsaturated, highly reactive, chlorinated cyclic hydrocarbon of low water solubility. HEX is a chemical intermediate in the manufacture of chlorinated pesticides and flame retardants with essentially no end uses of its own. The major source of environmental contamination by HEX is the aqueous discharge from production facilities, with small concentrations present as contaminants in commercial products made from it. However, HEX is not frequently found in the environment and, even when present, it is rapidly degraded. In view of this and recent controls on environmental emissions, current environmental exposure to HEX is extremely low. From time to time, isolated instances such as the sewer system disposal of HEX wastes (an illegal act) in 1977 in Louisville, KY, and the cleanup of a large waste disposal site in Michigan in 1983, have brought this chemical to the forefront of environmental news.

Hexachlorocyclopentadiene is not readily absorbed via epithelial tissues because it is highly reactive, especially with the contents of the gastrointestinal tract. HEX is moderately toxic when given orally, but has been estimated to be 100 times more toxic when inhaled. The data base for chronic toxicity of HEX is very limited. A chronic inhalation bioassay is being conducted by the National Toxicology Program (NTP) and may provide data regarding any carci- nogenic potential of HEX.

Several literature reviews on the health and environmental effects of HEX are available and include the following: Equitable Environmental Health, Inc. (1976), National Academy of Sciences (NAS, 1978), Bell et al. (1978) and U.S. EPA (1980c). Although each of these reports is different in scope and emphasis, a large amount of the scientific knowledge about HEX is included in these documents. To avoid unnecessary duplication, previously reviewed material found in these documents will not be considered at great length, except when it impinges directly upon present critical considerations. The information presented in this document is current through 1984, and contains a critical evaluation of some data which were not available at the publication time of the previously mentioned documents.

One final note of caution for the interested reader. Some of the reports reviewed in this document are unpublished laboratory reports. The Agency has received copies of these documents from various sources under the Toxic Substances Control Act (TSCA) reporting provisions. It is not the purpose of this document to judge the quality or validity of these reports unless there are peer review studies to compare results. The overall purpose of this document is to present the research data in order to assist the regulatory office of the Agency in developing a proposal concerning the decision to regulate HEX under Section 112 of the Clean Air Act.

The subject matter contained in this health assessment document has been reviewed by many Agency scientists, as well as scientists from private corporations, other government Agencies, and the general public. A previous draft of this publication was available for public comment. This final publication incorporates all of these comments and responses, as well as new literature published since the previous draft.

# 2. SUMMARY, CONCLUSIONS AND RESEARCH NEEDS

## 2.1. SUMMARY

**2.1.1. Properties, Production and Uses.** Hexachlorocyclopentadiene (HEX, C-56) is a dense pale-yellow or greenish-yellow, nonflammable liquid with a unique, pungent odor. HEX has a molecular weight of 272.79 and low water solubility. It is highly reactive and undergoes addition, substitution and Diels-Alder reactions.

Hexachlorocyclopentadiene is produced by only one company in the United States, Velsicol Chemical Corporation. Production data are considered proprietary; however, it has been estimated that between 8 and 15 million pounds/year are produced. HEX has been used as an intermediate in the production of many pesticides; however, this use has been limited by restrictions on the production of certain organochlorine pesticides. HEX is also used in the manufacture of flame retardants, resins and dyes.

**2.1.2. Sources, Environmental Levels, Transport and Fate.** HEX is released into the environment at low levels during its manufacture and during the manufacture of products requiring HEX. HEX can enter the environment in low levels as an impurity and contaminant in some of the products using HEX as an intermediate. There are only limited monitoring data available concerning the environmental levels of HEX. The available information suggests that HEX will be present mainly in the aquatic compartment and associated with bottom sediments and organic matter, with the exception of areas where land disposal has taken place. HEX readily adsorbs to most soil particles.

Releases of HEX to the atmosphere can result from the production and use of HEX, disposal of waste streams containing HEX, or from products contaminated with HEX. The total annual estimated release of HEX to the environment is 11.9 Mg (12.5 tons). Because of its physical and chemical characteristics, only a small amount of this total can be expected to persist.

The fate and transport of HEX in the atmosphere, considering available information, suggests that the compound has a tropospheric residence time (the time required for the concentration to be reduced to 1/e) of ~5 hours. However, atmospheric transport of HEX from an area of stored wastes and from wet wells during treatment of industrial wastes has been demonstrated.

In water, HEX may undergo photolysis, hydrolysis and biodegradation. In shallow water, HEX has a photolytic half-life of <1 hour. In deeper water where photolysis is precluded, the hydrolytic half-life of HEX is several days, while biodegradation is predicted to occur more slowly. HEX is known to volatilize from water, but this is influenced by turbulence and adsorption onto sediments.

HEX should be relatively immobile in soil based on its low water solubility. Volatilization, which is likely to occur primarily at the soil surface, is inversely related to the organic matter levels and water-holding capacity of the soil. Chemical hydrolysis and microbial metabolism are expected to reduce levels of HEX in soils.

Using model ecosystem data, the bioconcentration/bioaccumulation/biomagnification potential of HEX would theoretically be expected to be substantial based on its high lipophilicity [log octanol/water partition coefficient (log P)]. However, experimental evidence does not support this theory. Bioaccumulation factors derived from a short-term model ecosystem study appear to indicate a moderate accumulation potential in algae, snails, mosquito larvae, and mosquito fish. In addition, studies with laboratory animals have shown that HEX is excreted rapidly within the first few hours after oral dosing, with little being retained in the body. The compound did not biomagnify substantially from algae to snails or from mosquito larvae to fish. In addition, steady-state bioconcentration factors, measured in 30 to 32-day flow-through exposures, were only 29 and <11 in fish exposed to constant HEX levels of 20.9 μg/ℓ and 9.1 ppb, respectively. Therefore, it would appear from these data that HEX does not persist or accumulate in any large amounts. The degradation products of HEX have not been identified.

2.1.3. **Aquatic Life, Vegetation and Wildlife.** Low concentrations of HEX have been shown to be toxic to aquatic life. Lethality in acute (48- to 96-hour) exposures has been observed in both freshwater and saltwater crustaceans and fish at nominal concentrations of 32-180 μg/ℓ in static exposure systems in which the water was not renewed during the test. In the only studies using flowing water and measured HEX concentrations, identical 96-hour $LC_{50}$ values of 7 μg/ℓ were obtained for freshwater fish and saltwater shrimp. Chronic tests with the latter two species showed adverse effects at levels as low as 7.3 and 0.70 μg/ℓ, respectively.

Seven-day static tests with marine algae showed median reduction of growth ($EC_{50}$) at nominal concentrations ranging from 3.5-100 μg/ℓ, depending on the species.

In aqueous media, HEX is toxic to many microorganisms at nominal concentrations of 0.2-10 mg/ℓ, or levels substantially higher than those needed to kill most aquatic animals or plants. Some microorganisms are able to withstand HEX exposures as high as 1000 mg/ℓ. HEX appears to be less toxic to microorganisms in soil than in aquatic media, probably due to adsorption of HEX on the soil matrix.

Sufficient information is not available to determine the effects of HEX exposure on terrestrial vegetation or wildlife, although data from laboratory studies summarized in the following sections could be used to estimate effects on mammals in the wild.

**2.1.4. Pharmacokinetics, Toxicology, Exposure and Health Effects.** The absorption of unchanged HEX is lessened because of its reactivity with membranes and tissues, and especially with the contents of the gastrointestinal tract. HEX is considered a primary irritant, extremely toxic by inhalation, and moderately toxic by oral ingestion. Radiolabeled $^{14}C$-HEX is retained by the kidneys and liver of animals after oral or inhalation dosing; after inhalation, the trachea and lungs also retain radiolabeled material. Absorbed HEX is metabolized and rapidly excreted, predominantly in the urine and feces with <1% of the HEX found in expired air. Following inhalation or intravenous injection no unchanged HEX is excreted, and the fecal and urinary metabolites have been isolated, but not identified. The failure to identify these metabolites has been one of the mysteries concerning HEX. Without this information and quantitative data, it is difficult to assess the total effect of inhaled HEX in humans.

The acute inhalation lethal concentration ($LC_{50}$) of 1.6 and 3.5 ppm in male and female rats, respectively, has been demonstrated. Although there are some interspecies differences among guinea pigs, rabbits, rats and mice, HEX vapors are toxic to all species tested. HEX appears most toxic when administered by inhalation, with oral and then dermal administration being less toxic routes. Systemic effects of acute exposure include degenerative changes in the lungs, liver, kidneys and adrenal glands.

Subchronic oral dosing of rats (38 mg/kg/day) and mice (75 mg/kg/day) for 91 days produced nephrosis and inflammation and hyperplasia of the forestomach. No overt signs were noted when mice or rats were exposed by inhalation at 0.2 ppm of HEX (6 hours/day, 5 days/week) for 14 weeks.

However, inhalation exposure of rats at 0.5 ppm for 30 weeks caused degenerative changes in the liver, respiratory tract and kidneys. *In vitro* test results from three species have not shown HEX to be a mutagen. HEX was also inactive in the mouse dominant lethal assay.

Limited data are available on effects of exposure in humans. Isolated events have occurred which show HEX to cause severe irritation of the eyes, nose, throat and lungs. Human exposures have included short-term irritations, with recovery after cessation of exposure. There were no statistically significant differences in liver enzymes between exposed and control groups. The long-term health effects of continuous low-level exposure and/or intermittent acute exposure in man are not known. Waste handlers and sewage workers have been shown to be occupations at risk.

The data base is neither extensive nor adequate for assessing the carcinogenicity of HEX. The National Toxicology Program (NTP) has recently completed a subchronic animal study and will begin a lifetime animal inhalation bioassay using both rats and mice. Several epidemiologic studies were cited in the literature; however, no increased incidences of neoplasms at any site were reported which could be related to HEX. Accordingly, Velsicol Chemical Corporation has on-going programs and follow-up studies in order to study the long-term effects of HEX exposure. A final judgment of carcinogenicity will have to be deferred until the results of the NTP bioassay are available. Using the International Agency for Research on Cancer (IARC) criteria, the available evidence matches the overall Group 3 category. According to the IARC criteria, Group 3 indicates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect.

## 2.2. CONCLUSIONS

This document presents the current scientific data base concerning HEX. HEX is not found frequently in the environment because its emissions are low (~12 megagrams per year) and because it rapidly degrades into other substances of unknown character. This document summarizes the known health effects from exposure to HEX. At expected ambient concentrations, there have been no known long-term adverse health effects. The only known effect of HEX that might occur at current and projected exposures is odor recognition. The odor recognition threshold concentration for HEX, which is not well-established, may be exceeded in the vicinity of the sources listed in this chapter. At this time, available information is not sufficient from either animal or human data to determine the carcinogenic potential of HEX. In addition, as listed below, there are still uncertainties in the data base that affect the interpretation of available data. Once these voids in data are filled, we will have a better understanding of HEX and its effect on humans and the environment.

## 2.3. RESEARCH NEEDS

In the development of this document and previous drafts, there have been many comments on the need to complete certain studies. This data would refine the known information and give scientists a better understanding of the effects of HEX and its properties. Because studies on the carcinogenic potential of HEX are being done by NTP, additional research for carcinogenicity does not appear to be warranted. In its response to the Interagency Testing Committee regarding section 4 of TSCA, the Agency stated that, given current manufacture, distribution in commerce, use or disposal of HEX, there was no need to acquire more test data to make regulatory decisions under TSCA. However, as the result of this document and its review, the following research areas could yield data that would provide information on the specific nature of this compound, as well as help resolve some remaining unknowns.

- An unresolved issue at the peer review workshop concerned the matter in which external factors influence the vapor pressure of HEX. Considerable discussion resulted in the recommendation that a study of vapor pressure should be included as a priority item in future research.
- There is a need for a thorough metabolism study in which the metabolites are isolated and identified.
- Monitoring and study of groups exposed to continuous low levels of HEX is warranted. Monitoring data are needed to derive estimates of exposure, especially for those areas near production and formulation facilities.
- Further studies are needed to determine the fate of HEX in the environment.
- Teratogenicity studies should be conducted using various routes of exposure, with emphasis on the inhalation route.
- There is a need to measure the odor recognition threshold of HEX. One study has been performed; however, this study was not peer reviewed.

# 3. PHYSICAL AND CHEMICAL PROPERTIES/ANALYTICAL METHODOLOGY

## 3.1. SYNONYMS, TRADE NAMES AND IDENTIFICATION

Hexachlorocyclopentadiene (HEX) is the most commonly used name for the compound that is designated 1,2,3,4,5,5'-hexachloro-1,3-cyclopentadiene by the International Union of Pure and Applied Chemistry (IUPAC) system.

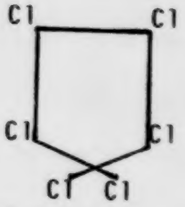
Table 3-1 cites the IUPAC name and synonyms, identification numbers and molecular and structural formulas of HEX.

## 3.2. PHYSICAL AND CHEMICAL PROPERTIES

**3.2.1. Physical Properties.** Hexachlorocyclopentadiene is a nonflammable liquid with a characteristic pungent, musty odor; the pure compound is light lemon-yellow. Impure HEX may have a greenish tinge (Stevens, 1979). It has a molecular weight of 272.79 and its molecular formula is $C_5Cl_6$. Hexachlorocyclopentadiene (98%) is a dense liquid (sp. gr. 1.7019 at 25°C) with low solubility in water (0.805-2.1 mg/ℓ at 25°C). A detailed list of physical properties is presented in Table 3-2. The compound is strongly adsorbed by soil colloids. It volatilizes rapidly from water (Atallah et al., 1980). According to the Handbook of Chemistry and Physics (Weast and Astle, 1980), the ultraviolet visible $\lambda_{max}$ in heptane is 323 nm with a log (molar absorptivity) of 3.2. This absorption band reaches into the visible spectrum, as evidenced by the yellow color of HEX. Facile carbon-chlorine bond scission might be expected in sunlight or under fluorescent light. The IR spectrum has characteristic absorptions at 6.2, 8.1, 8.4, 8.8, 12.4, 14.1 and 14.7 μm. The mass spectrum of HEX shows a weak molecular ion (M) at M/e 270, but a very intense (M-35) ion making this latter ion suitable for sensitive specific ion monitoring.

TABLE 3-1

Identity of Hexachlorocyclopentadiene

| Identifying Characteristic | Name/Number/Structure |
|---|---|
| IUPAC Name: | 1,2,3,4,5,5'-Hexachloro-1,3-cyclopentadiene |
| Trade Names: | C56; HRS 1655; Graphlox |
| Synonyms: | Hexachlorocyclopentadiene<br>Perchlorocyclopentadiene<br>HEX<br>HCPD<br>HCCP<br>HCCPD<br>C-56<br>HRS 1655<br>Graphlox |
| CAS Number | 77-47-4 |
| CIS Accession Number: | 7800117 |
| Molecular Formula: | $C_5Cl_6$ |
| Molecular Structure: |  |

## TABLE 3-2

## Physical Properties of Hexachlorocyclopentadiene

| Property | Value/Description | Reference |
|---|---|---|
| Molecular Weight: | 272.79 | Stevens, 1979 |
| Physical Form (25°C) | Pale yellow liquid | Hawley, 1977; Irish, 1963 |
| Odor: | Pungent | Hawley, 1977; Irish, 1963 |
| Electronic Absorption Maximum [(in 50% acetonitrile-water)] | 322 nm | Wolfe et al., 1982 |
| Solubility (25°C) | | |
| Water (mg/ℓ): | 2.1 | Dal Monte and Yu, 1977 |
| | 0.805 | Lu et al., 1975 |
| | 1.8 (28°C) | Wolfe et al., 1982 |
| Organic Solvents: | Miscible (Hexane) | Bell et al., 1978 |
| Vapor Density (air = 1) | 9.4 | Verschueren, 1977 |
| Vapor Pressure (mmHg, °C): | 0.08 (25°C) | Irish, 1963 |
| | 0.975 (62°C) | Stevens, 1979 |
| Specific Gravity: | 1.717 (15°C) | Hawley, 1977 |
| | 1.710 (20°C) | Stevens, 1979 |
| | 1.7019 (25°C) | Weast and Astle, 1980 |
| Melting Point (°C): | -9.6 | Hawley, 1977 |
| | -11.34 | Stevens, 1979 |
| Boiling Point (°C): | 239 @ 753 mm Hg | Hawley, 1977; Stevens, 1979 |
| | 234 | Irish, 1963 |
| Octanol/Water Partition Coefficient (log P) | | |
| (measured): | $5.04 \pm 0.04$ | Wolfe et al., 1982 |
| (estimated): | 5.51 | Wolfe et al., 1982 |
| Latent Heat of Vaporization | 176.6 J/g | Stevens, 1979 |
| Henry's Law Constant (atm-m$^3$/mole) | $2.7 \times 10^{-2}$ | Atallah et al., 1980; Wolfe et al., 1982 |

**3.2.2. Chemical Properties.** Commercial HEX has various purities depending upon the method of synthesis. HEX made by chlorination of cyclopentadiene by alkaline hypochlorite at 40°C, followed by fractional distillation, is only 75% pure, and contains many lower chlorinated cyclopentadienes. Purities >90% have been obtained by thermal dechlorination of octachlorocyclopentene at 470-480°C (Stevens, 1979). The current specification for HEX produced by Velsicol Chemical Corporation at the Memphis, TN plant, which is used internally and sold to other users, has a 97% minimum purity (Velsicol Chemical Corporation, 1984).

If moisture is excluded, HEX can be stored without harming the product or its containers. Storage containers should not have iron in their inner linings (Stevens, 1979).

Hexachlorocyclopentadiene is a highly reactive diene that readily undergoes addition and substitution reactions and also participates in Diels-Alder reactions (Ungnade and McBee, 1958). The products of the Diels-Alder reaction of HEX with a compound containing a non-conjugated double bond are generally 1:1 adducts containing a hexachlorobicyclo(2,2,1)heptene structure; the monoene derived part of the adduct is nearly always in the endo-position, rather than the exo-position (Stevens, 1979). Figure 3-1 illustrates synthetic pathways to various chlorinated pesticides for which HEX is a precursor. Flame retardant chemicals for which HEX is a precursor include chlorendic acid, chlorendic anhydride and Dechlorane Plus (Stevens, 1979).

Two excellent early reviews of the chemistry of HEX were published by Roberts (1958) and Ungnade and McBee (1958). Look (1974) reviewed the formation of HEX adducts of aromatic compounds and the by-products of the Diels Alder reaction.

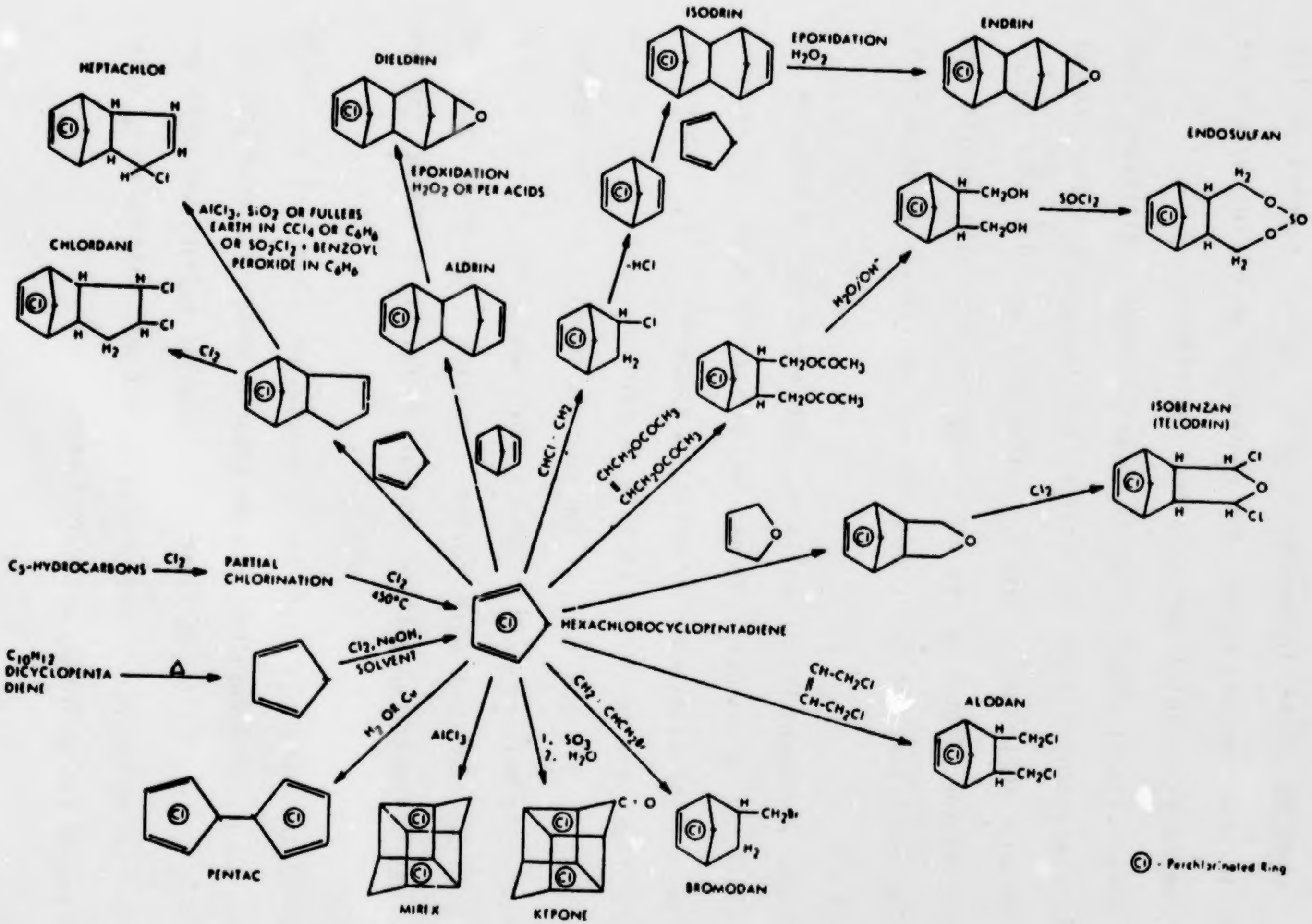

HEPTACHLOR
DIELDRIN
ISODRIN
ENDRIN
ENDOSULFAN
CHLORDANE
ALDRIN
EPOXIDATION
H2O2 OR PER ACIDS
EPOXIDATION
H2O2
AlCl3, SiO2 OR FULLERS EARTH IN CCl4 OR C6H6 OR SO2Cl2 + BENZOYL PEROXIDE IN C6H6
Cl2
-HCl
H2O/OH-
SOCl2
CHCl : CH2
CHCH2OCOCH3 ‖ CHCH2OCOCH3
CH2OCOCH3
CH2OH
ISOBENZAN (TELODRIN)
Cl2
C5-HYDROCARBONS
PARTIAL CHLORINATION
Cl2 450°C
HEXACHLOROCYCLOPENTADIENE
C10H12 DICYCLOPENTADIENE
Cl2, NaOH, SOLVENT
CH-CH2Cl ‖ CH-CH2Cl
ALODAN
H2 OR Cu
AlCl3
1. SO3 2. H2O
CH2 : CHCH2Br
PENTAC
MIREX
KEPONE
BROMODAN
C : O
CH2Br
CH2Cl
Cl - Perchlorinated Ring

FIGURE 3-1

Synthesis of Chlorinated Cyclodiene Pesticides from Hexachlorocyclopentadiene

Source: Adapted from Lawless et al., 1972; Bell et al., 1978

## 3.3. ANALYTICAL METHODOLOGY

### 3.3.1. Air.

**3.3.1.1. SAMPLING** -- The techniques used to collect samples of HEX vapor in air involve the adsorption and concentration of the vapors in liquid-filled impingers or solid sorbent-packed cartridges.

Whitmore et al. (1977) pumped airborne vapors through a miniature glass impinger tube containing hexane or benzene and through a solid sorbent packed (Chromosorb® 102) tube. Sampling efficiency was 97% with hexane and ~100% with benzene. The sampling efficiency for the solid sorbent tube was ~100%. The sensitivity of the liquid impinger system was found to be <1 ppb in ambient air.

Kominsky and Wisseman (1978) collected HEX vapor on Chromosorb® 102 (20/40 mesh) sorbent previously cleaned by extraction with 1:1 acetone/methanol. The extraction removed interfering compounds. The sorbent was packed into a front 100-mg and a back 50-mg section separated by a 2 mm polyurethane plug in a glass tube, 7 cm long and 4 mm i.d. The samplers were collected using battery powered vacuum pumps operating at 0.05 or 0.20 ℓ/minute. HEX was desorbed with carbon disulfide (68% efficiency) and analyzed by gas chromatography-flame ionization detection (Neumeister and Kurimo, 1978).

In studying the pyrolysis products of endosulfan, Chopra et al. (1978) collected the vapors of endosulfan-treated tobacco smoke in a cold trap containing pentane cooled to 0 and -80°C. The pentane extract was then prepared for gas chromatographic (GC) analysis; HEX was qualitatively determined to be one of the pyrolysis products formed.

Under contract with NIOSH, Boyd et al. (1981) and Dillon (1980) of the Southern Research Institute developed and validated sampling and analytical methods for air samples containing HEX. Methods were reliable below the 8-hour time-weighted-average (TWA) Threshold Limit Value (TLV) of 0.1 $mg/m^3$ recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).

The developed NIOSH method, P & CAM 308 (NIOSH, 1979) utilized adsorption on Porapak® T (80/100 mesh), desorption with hexane (100% for 30 ng of HEX on 50-100 mg adsorbent), and then analysis by GC-$^{63}$Ni electron capture detection. The solid sorbent was cleaned by soxhlet extraction with 4:1 (v/v) acetone/methanol (4 hours), and hexane (4 hours), and was allowed to dry under vacuum at 50-70°C overnight before cooling in a desiccator. The pyrex sampling tubes (7 cm long, 6 mm o.d., 4 mm i.d.) contained a front 75 mg layer of sorbent and a 25 mg backup section. Each section was held in place with two silylated glass wool plugs. A 5 mm long airspace was necessary between the front and backup sections. A battery operated sampling pump drawing air at 0.05 and 2.0 ℓ/minute was utilized for personal sampling of workers. The lowest analytically quantifiable level was 25 ng HEX/sorbent sample, assuming 1 mℓ of hexane-desorbing solvent and a 1 hour desorption time by ultrasonification. The upper limit of the method was 2500 ng/sorbent sample. The method was validated for air HEX concentrations between 13 and 865 $\mu g/m^3$ at 25-28°C and a relative humidity of $\geq 90\%$.

3.3.1.2. **ANALYSIS** -- Gas chromatography is the preferred method for analyzing HEX in air using either flame ionization collection or electron capture detection (e.g., Chopra et al., 1978; Neumeister and Kurimo, 1978; Whitmore et al., 1977; NIOSH, 1979). Gas chromatography/mass spectroscopy (GC/MS) is necessary for confirmation (Eichler, 1978).

Several sorbent materials were evaluated for collection of HEX vapor: Amberlite® XAD-2 (20/50 mesh), Porapak® R (50/80 mesh), Ambersorb® XE-340 (20/50 mesh), Chromosorb® 104 (60/80 mesh), Tenax-GC® (35/60 mesh), Porapak® T (80/100 mesh) and Porapak® T (50/80 mesh). According to the NIOSH criterion for acceptable methods, a sorbent material must have a demonstrated sorption capacity for the analyte that is adequate for sampling a reasonable volume of workplace air at an established rate. Table 3-3 enumerates additional factors related to the Porapak® T collection system.

Gas chromatography with electron capture detection (ECD) was determined to be the most sensitive analytical technique. For HEX the chromatographic response was stated to be a linear and reproducible function of HEX concentration in the range of ~5-142 ng/mℓ (25-710 pg injected) with a correlation coefficient of 0.9993 for peak height measurement. The optimized operating conditions for this method are shown in Table 3-4.

Validation tests were conducted according to NIOSH guidelines. The accuracy and precision of the overall sampling and analytical procedure for HEX were evaluated in the concentration range of ~13-865 μg/m³. The lowest analytically quantifiable level (LAQL) of HEX was determined to be 25 ng/sorbent tube. This level represents the smallest amount of HEX that can be determined with a recovery of >80% and a relative standard deviation (RSD) of <10%. The desorption efficiency of 100% was determined by averaging the levels ranging from near the LAQL of 25 ng to 1000 times the LAQL.

3.3.2. **Water.** Since HEX is sensitive to light in both organic and aqueous solutions, the water samples, extracts and standard HEX solutions must be protected from light. The rate of degradation is dependent upon the intensity and wavelength with the half-life of HEX being ~7 days when the

TABLE 3-3

Characteristics of the Porapak® T Collection System[a]

| Characteristic | HEX Type/Value |
|---|---|
| Sorbent material | Porapak® T[b]<br>(80/100 mesh) |
| Breakthrough time[c] | >8 hour (0.2 ℓ/minute) |
| Breakthrough volume[c] | >100 ℓ |
| Tube capacity[c] | >100 g |
| Average desorption efficiency of indicated quantity of analyte | 0.94 (27.4 ng) |
| Sorbent tube configuration[d] | 75 mg sorbing layer,<br>25 mg backup layer |
| Extraction solvent | Hexane |

[a] Adapted from Boyd et al., 1981

[b] This material required cleaning by Soxhlet extraction (see text).

[c] For these tests the temperature of the generator effluent was maintained at 25-28°C and the relative humidity at >90%. The concentration of the analyte in the generator effluent was 1 mg/m³ of HEX.

[d] The sorbent tubes were Pyrex (7 cm long by 6 mm o.d. and 4 mm i.d.). Silanized glass wool plugs separated the sections.

## TABLE 3-4

### Optimized GC Analytical Procedure for HEX[a],[b]

| Characteristic | Type/Value |
|---|---|
| Detector | Electron capture |
| Column | 3% OV-1 on Gas-Chrom Q (100/120 mesh) in glass (4 mm i.d. by 2 m) |
| **OPERATING CONDITIONS** | |
| Carrier gas (20 mℓ/minute) | 5% $CH_4$, 95% Ar |
| Temperatures | |
| Injection port | 150°C |
| Column | 135°C |
| Detector | 250°C |
| Detector parameters | Detector purge, 5% $CH_4$ with 95% Ar (80 mℓ/minute) |
| Solvent for compound[c] | Hexane |

[a] Adapted from Boyd et al., 1981

[b] A Hewlett-Packard 5750A gas chromatograph was used.

[c] The injection volume was 5 μℓ of sample and 1 μℓ of solvent flush.

solution was exposed to ordinary laboratory lighting conditions (Benoit and Williams, 1981). Storing the HEX-containing solutions in amber or red (low actinic) colored glassware is recommended for adequate protection (Benoit and Williams, 1981).

The XAD-2 resin extraction has been used to concentrate HEX from large volumes of water. Solvent extraction of water has also proved successful. The detection limit used for the organic solvent extraction technique was 50 ng/ℓ vs. 0.5 ng/ℓ for the XAD-2 method. Using the solvent extraction method under subdued laboratory lighting conditions, the efficiency of recovery for an artificially loaded water sample was in the range of 79-88%. The authors concluded that the XAD-2 resin could not be used to accurately sample HEX in water but could be used to screen samples qualitatively because of the low detection limit (Benoit and Williams, 1981).

### 3.3.3. Soil.

3.3.3.1. **SAMPLING** -- In the method described by DeLeon et al. (1980a), samples were taken from vertical borings 30 feet deep using the split-spoon method. The samples were then placed in jars and sealed with Teflon®-lined screw caps. During shipment, the samples were maintained at 6-10°C. Upon their arrival at the analysis site, they were maintained at -20°C until required for analysis.

3.3.3.2. **ANAYLSIS** -- DeLeon et al. (1980a) developed a method for determining volatile and semivolatile organochlorine compounds in soil and chemical waste disposal site samples. This procedure involves hexane extraction followed by analysis of the extract by temperature-programmed gas chromatography on high-resolution glass capillary columns using electron capture detection; GC/MS is used for confirmation of the presence of the chlorocarbons. The method has a lower detection limit of 10 μg/g.

Spiked samples of soil were used to test the recovery and reproducibility of the procedure. When a soil sample was spiked with a 10 µg/g concentration of HEX, the recovery was 59.8% (S.D. 6.1); at 100 µg/g, 95.9% (S.D. 15.9); at 300 µg/g, 90.2% (S.D. 4.1). However, as the authors state, some modifications may be necessary for analysis of the more volatile one to four carbon chlorinated compounds, since some compounds may be lost in the concentration step before GC analysis. Of the 11 compounds tested in three different concentration levels by the authors, the 100 µg/g HEX sample had the highest standard deviation of all compounds. Over 100 chemical waste disposal site and soil samples have been evaluated by this method. In this study, HEX was not detected in three typical samples each taken from a different location within and around a chemical waste disposal site (DeLeon et al., 1980a).

## 3.4. BIOLOGICAL MEDIA

**3.4.1. Sampling.** A method to determine levels of HEX in blood and urine has been described by DeLeon et al. (1980b). This method involves isolation of the compound from the blood or urine sample by liquid-liquid extraction, GC analysis with electron capture detection and confirmation by GC/MS. Mean recoveries of 28.8 and 54.5% were reported for blood samples containing 50 and 500 ng/mℓ, respectively; for urine, mean recoveries of 35.0 and 51.8% were reported for samples containing 10 and 200 ng/mℓ, respectively. The best recoveries were obtained in the study through the use of a toluene-acetonitrile extraction combination for blood assays, and petroleum ether extraction for urine assays. The authors concluded that this method is useful for the detection and identification of nanogram quantities of HEX, with low detection limits of 50 ng/mℓ for blood and 10 ng/mℓ for urine.

Studies by Velsicol Chemical Corporation have shown that up to 30% of the HEX can be lost if the extracts are concentrated to 0.1 mℓ. Quantitative recovery was possible only for volumes of concentrate >0.5 mℓ. This limits the sensitivity of the method. However, the method may offer a sensitive means of monitoring occupational exposure.

**3.4.2. Analysis.** Velsicol Chemical Corporation (1979) has developed three analytical methods which have been used for urine, fish fillet, beef liver, beef skeletal muscle, beef adipose tissue, beef kidney, chicken liver, chicken skeletal muscle and chicken adipose tissue. The respective recoveries were: 80±10 (1-50 ppb), 81±1, 69±4, 88±2, 86±5, 71±3, 55±9, 76±4 and 85±2%. The level of fortification for the tissue samples was 10 ppb. For urine, up to 31% HEX could be degraded when the fortified urine sample was stored overnight in a cooler.

Urine was extracted with hexane, the hexane passed through anhydrous sodium sulfate, and evaporated to 1 mℓ. The limit of detection for HEX without concentrating the extract was 0.5 ppb. For cattle, poultry and fish tissues, the tissues were extracted with 2:1 pentane/acetone, the homogenate diluted with 10% sodium chloride solution, centrifuged, and the pentane layer transferred into a separatory funnel. The residues were then partitioned into acetonitrile (3 times), water diluent added to the acetonitrile, and then back-extracted with pentane. The pentane extract was treated with concentrated sulfuric acid and then water, and concentrated to ~3 mℓ. Upon dilution to 10 mℓ with hexane, the solution was treated with a 1:1 concentrated sulfuric acid/fuming sulfuric acid solution, water, and a 9:1 mixture (solid) of sodium sulfate/sodium carbonate. Packed columns (3% OV-1 on Gas Chrom Q-100/120 mesh-in 2 m x 2 mm i.d. glass column) or capillary columns (30 m x 0.25 mm SE-30 WCOT) can be used for GC using a $^{63}$Ni-electron capture detector.

# 4. PRODUCTION, USE, SOURCES AND AMBIENT LEVELS

## 4.1. PRODUCTION

Because there is only one producer of HEX, production statistics are considered confidential business information (CBI) and are not available to the general public. Production estimates for HEX, based on the manufacture of chlorinated cyclodiene pesticides in the early 1970s, were ~50 million pounds per year (Lu et al., 1975). Following restrictions in the use of pesticides produced from HEX, production estimates were lowered to a range of 8-15 million pounds per year (U.S. EPA, 1977). In a report prepared for the U.S. EPA, Hunt and Brooks (1984) estimated 8300 Mg (9130 tons) of HEX were produced in 1983. Technical grade HEX usually contains other chemicals as contaminants of manufacture (e.g., hexachlorobenzene and octachlorocyclopentene. The nature and levels of contaminants will vary with the method of production.

## 4.2. USE

HEX is the key intermediate in the manufacture of some chlorinated cyclodiene pesticides (see Figure 3-1). These include heptachlor, chlordane, aldrin, dieldrin, endrin, mirex, PENTAC and endosulfan. Another major use of HEX is in the manufacture of flame retardants such as chlorendic anhydride and Dechlorane Plus. HEX is also used to a lesser extent in the manufacture of resins and dyes (U.S. EPA, 1980c), and has been used previously as a general biocide (Cole, 1954). Currently, HEX is produced at two locations: Memphis, TN and Marshall, IL. All of the HEX produced at the Illinois plant is used solely for the production of chlordane, and is not sold or distributed, while that produced at the Memphis plant is used to produce heptachlor, endrin and the fire-retardant chlorendic anhydride. The HEX produced at the Memphis plant is the same as that sold in commerce to users of HEX (Velsicol Chemical Corporation, 1984).

## 4.3. SOURCES

HEX is released into the environment in low levels during its manufacture and during the manufacture of products requiring HEX (U.S. EPA, 1980c). It is also found as an impurity and a degradation product in compounds manufactured from HEX (Spehar et al., 1977; Chopra et al., 1978). Limited monitoring data from production sites indicated that HEX was present at 18 mg/ℓ (on February 2, 1977) in the aqueous discharge from the Memphis pesticide plant (U.S. EPA, 1980c). In the summer of 1977, shortly after these readings, a new wastewater treatment plant began operation. Before construction of the plant, wastewater flowed directly into the Mississippi River or through one of its tributaries (Elia et al., 1983). Voluntary improvements in controlling the discharge from the Memphis plant resulted in reported levels of 0.07 ppb HEX in the Mississippi River, near the mouth of Wolf Creek (Velsicol Chemical Corp., 1978). HEX measurements were taken from the effluent stream of the Memphis North Sewage Treatment Plant from February to July 1982. Monthly averages ranging from 0.15-0.61 ppb were reported. Table 4-1 summarizes these data (Levin, 1982b). In May 1977, HEX was also detected at 0.17 mg/ℓ in the aqueous discharge and at 56 ppb in air samples collected from a waste site in Montague, MI (U.S. EPA, 1980c). Indoor air concentrations of HEX in some Tennessee houses with contaminated groundwater supplies ranged from 0.06 to 0.10 μg/m³ (S. Clark et al., 1982). HEX has also been identified in the soil and river sediments downstream from a Virginia manufacturing plant, even after pesticide production was discontinued (U.S. EPA, 1980c). Under contract with the U.S. EPA, the Radian Corporation prepared a report which presented the results of a preliminary source assessment on HEX (Hunt and Brooks, 1984). Some of the results of this study are presented in the following sections.

TABLE 4-1

HEX Content in the Effluent Stream
of the Memphis North Sewage Treatment Plant, 1982[a]

| Month | Number of Samples Analyzed | HEX Level (ppb) | | |
|---|---|---|---|---|
| | | High | Low | Average[b] |
| February | 19 | 0.80 | ND[c] | 0.32 |
| March | 15 | 0.60 | ND[c] | 0.34 |
| April | 30 | 3.04 | ND[c] | 0.61 |
| May | 31 | 0.54 | ND[c] | 0.24 |
| June | 29 | 0.57 | ND[c] | 0.18 |
| July | 30 | 1.80 | ND[c] | 0.15 |

[a] Source: Levin, 1982b

[b] Average of all samples taking all ND (not detected) values as zero.

[c] Detection limit is <0.01 ppb

## 4.4. AMBIENT LEVELS

Published reports, environmental releases and physicochemical properties of HEX imply that it will be present mainly in the aquatic compartment and associated with bottom sediments and organic matter. Relatively much lower concentrations will be found in the soil and air compartments.

**4.4.1. Air.** Releases of HEX to the atmosphere can result from the production and use of HEX, disposal of waste streams containing HEX or from products contaminated with HEX (Hunt and Brooks, 1984). Data sent to the U.S. EPA regarding emission levels from Velsicol plants indicate that quantities of HEX are emitted into the air; however, these data are not considered public information. No data were found that reported ambient atmospheric levels of HEX; however, the half-life of HEX in air is <5 hours (Cupitt, 1980), which greatly reduces the potential for measurement. The highest reported concentration of HEX measured in Tennessee homes was 0.10 μg/m³, while air levels at the Memphis North Treatment plant ranged as high as 39 μg/m³ (S. Clark et al., 1982; Elia et al., 1983). A list of values is given in Table 4-2 for these air samples. This plant handles the wastewater from a pesticide manufacturer five miles away. The only other air monitoring was done on an abandoned waste site in Michigan where the average HEX emission rate was 0.26 g/hr (±0.05).

**4.4.2. Water.** Environmental monitoring data for HEX are available from a number of sources. The bulk of the reported levels are contained within the STORET data base (U.S. EPA, 1980b). The available monitoring data (STORET) do not provide specific information about the sampling site and analytical methodology. Additionally, the STORET data has not been verified and it is not possible, therefore, to analyze the reported data critically.

## TABLE 4-2

### Area Air Samples Collected at the Memphis North Treatment Plan, 1978[a]

| Date | N[c] | Concentration[b], µg/m³ | | | |
|---|---|---|---|---|---|
| | | HEX | HEX-BCH | HCBCH[d] | Chlordene |
| **A. WET WELL** | | | | | |
| May | 3 | 0.03 | 219 | 87 | 45 |
| June | 2 | 18 | 278 | 15 | 16 |
| September | 2 | 8 | 25 | 200 | 44 |
| October | 1 | 15 | 2 | 1 | 0.1 |
| November | 1 | 39 | 7 | 85 | 7.8 |
| **B. GRIT CHAMBER** | | | | | |
| May | 3 | 0.03 | 4.1 | 1.9 | 0.9 |
| June | 7 | 1.9 | 6.5 | 1.7 | 7.5 |
| July | 2 | 0.03 | 0.5 | 0.7 | 2.3 |
| September | 4 | 0.03 | 0.5 | 1.1 | 2.7 |
| October | 1 | 0.04 | 1.2 | 1.0 | 0.8 |
| November | 1 | 12 | 2.6 | 4.3 | 1.0 |

[a] Source: Elia et al., 1983

[b] Mean values of the number of samples, N, indicated

[c] N designates the number of samples collected

[d] Heptachlorobicycloheptene

As previously mentioned, water samples of the influent wastewater were taken at the Memphis North Treatment plant (Table 4-3). However, in the S. Clark et al. (1982) study, HEX was not detected in the private wells of the Tennessee homes.

Benoit and Williams (1981) sampled both raw and drinking waters from an Ottawa water treatment plant. Using solvent extraction analysis with a detection sensitivity of 50 ng/ℓ or using the XAD-2 resin extraction method with a detection sensitivity of ~0.5 ng/ℓ no HEX was detected in the raw water, but levels ranging from 57-110 ng/ℓ were reported in the finished drinking water, suggesting that HEX was introduced into the drinking water during the treatment process. The authors did not find the source of the HEX, and are investigating their findings further (Benoit, 1983).

**4.4.3. Food.** HEX was qualitatively detected in fish samples taken from water near a pesticide manufacturing in Michigan (Spehar et al., 1977); however, none has been detected in fish samples taken from the waters near the pesticide manufacturing plant in Memphis (Velsicol Chemical Corp., 1978; Bennett, 1982). No information was available regarding HEX contamination of other foods.

**4.4.4. Soil.** Ambient monitoring data for the terrestrial environment are not available. However, it appears that these concentrations should be much lower than concentrations present in the aquatic environment. Depositing of HEX from the atmospheric (and aquatic) compartment into the terrestrial environment is expected to be minimal. Similarly, direct release of HEX into the terrestrial environment (i.e., as an impurity in chlorinated pesticides) should be decreasing with the possible exceptions of disposal at waste sites, accidental spills and other illegal disposal methods.

## TABLE 4-3

### Concentrations of Selected Organic Compounds in Influent Wastewater at Memphis North Treatment Plant, 1978[a]

| Date | N[c] | Concentration[b], μg/ℓ | | | |
|---|---|---|---|---|---|
| | | HEX | HEX-BCH | HCBCH[d] | Chlordene |
| June | 1 | 3 | 334 | 57 | 87 |
| August[e] | 5 | 0.8 | 329 | 115 | 216 |
| September | 2 | 4 | 292 | 668 | 58 |
| October-November | 2 | 0.8 | 11 | 17 | 32 |

[a] Source: Elia et al., 1983

[b] Mean values for the number of samples indicated

[c] Number of samples

[d] Heptachlorobicycloheptene

[e] These values are furnished by the chemist at the North plant.

## 4.5. RELATIVE SOURCE CONTRIBUTIONS

Available data are insufficient to derive relative source contributions. After considering the available information, the U.S. EPA has reported that human exposure through the environment via air or water would be extremely low except for workers and residents near manufacturing, shipping and waste sites, and concluded that exposure was not considered significant or substantial (U.S. EPA, 1982).

The only other estimation of relative source contributions is the Radian report previously mentioned (Hunt and Brooks, 1984). The air releases from manufacturing processes can be from vents on reactors, process and storage tanks, and fugitive emissions. Hunt and Brooks (1984) estimated the total quantity of HEX released from these sources to be 8.0 Mg (8.8 tons). In addition, HEX can be emitted to the air from the incineration and landfilling of wastes containing HEX, with the best estimation being 1.0 Mg (1.1 tons). The other sources include those listed in Section 4.4.4. and other discharges to water bodies. The total annual estimated release of HEX to the environment is 11.9 Mg (12.5 tons). These are only estimates because of the limited data and are given only to provide the relative magnitude of HEX emissions to the environment. For the reader who wishes to examine these data and assumptions further, the Radian report should be reviewed in its entirety.

## 4.6. SUMMARY AND CONCLUSIONS

Measured ambient concentrations of HEX are available for aquatic compartments (U.S. EPA, 1980b). These include freshwater and sediments of streams, lakes and wells. Limited data are also available for estuaries and oceans. Additional saltwater, as well as atmospheric and terrestrial monitoring data, are needed to determine the ambient concentrations in these compartments.

Freshwater levels of HEX are estimated to range from 0-800 μg/ℓ, based upon non-verified STORET data. Estimates for atmospheric concentrations are not available in the literature, while estimates for HEX concentrations in soils are limited. To achieve proper conclusions concerning the levels of HEX in the environment, careful monitoring and analysis must be conducted. To date, this information is very limited.

Air HEX levels in areas near previous dump sites have been shown to be high. High concentrations of HEX have been recorded in wastewater and in two incidents have increased the ambient HEX levels inside treatment facilities above the ACGIH time-weighted-average.

# 5. ENVIRONMENTAL FATE AND TRANSPORT

## 5.1. FATE

The evidence presented in this section indicates that HEX is not persistent in the air, water or soil. Photolysis, hydrolysis and biodegradation have been shown to be the key processes influencing the environmental fate of HEX.

**5.1.1. Air.** Little relevant information is available to predict the fate of HEX in air. Its tropospheric residence time was estimated by Cupitt (1980) to be ~5 hours based on estimated rates of reaction with hydroxyl radicals and ozone. The respective reaction rates were theoretically calculated to be $59\times10^{-12}$ and $8\times10^{-18}$ $cm^3$ $molecule^{-1}$ $sec^{-1}$. In estimating the tropospheric residence time, or time for a quantity of HEX to be reduced to 1/e (or ~37%) of its original value, it was assumed that the rate constants calculated at room temperature for both reactions are valid in the ambient atmosphere and that the background concentrations of hydroxyl radical and ozone are $10^6$ and $10^{12}$ molecules $cm^{-3}$, respectively. Atmospheric photolysis of HEX was also rated as "probable", since HEX has a chromophore that absorbs light in the solar spectral region, and is known to photolyze in aqueous media (see Section 5.1.2.1.). No attempt was made to estimate a rate for atmospheric photolysis. Cupitt (1980) listed the theoretical degradation products as phosgene ($Cl_2CO$), diacylchlorides, ketones and free chlorine (Cl-) radical, all of which would be likely to react with other elements and compounds.

Korte (1978) demonstrated the photomineralization of HEX (1.9 g) applied to silica gel (400 g) after 4 days irradiaton (λ > 290) in an atmosphere of pure oxygen. The mineralization products were chloride ($Cl^-$, 44.9%), carbon dioxide ($CO_2$, 48.3%), chlorine gas ($Cl_2$, 5.4%) and carbon monoxide (CO, 1.2%).

5.1.2. **Water.** In the event of release into shallow or flowing bodies of water, degradative processes such as photolysis, hydrolysis and biodegradation, as well as transport processes involving volatilization and other physical loss mechanisms, are expected to be prominent in HEX dissipation. In deeper, nonflowing bodies of water, hydrolysis and biodegradation may become the predominant fate processes.

5.1.2.1. **PHOTOTRANSFORMATION** -- Zepp et al. (1979) and Wolfe et al. (1982) reported the results of U.S. EPA studies on the rate of HEX phototransformation in water. Under a variety of sunlight conditions, in both distilled and natural waters of 1-4 cm depth, phototransformation half-life was <10 minutes. Addition of natural sediments to distilled water containing HEX had little effect on phototransformation rate. These findings indicated that the dominant mechanism of HEX phototransformation was direct absorption of light by the chemical, rather than photosensitization reactions involving other dissolved or suspended materials.

The direct photoreaction of HEX in water was also studied under controlled conditions in the laboratory using a monochromatic light (313 nm) isolated by filters from a mercury lamp. Phototransformation rate constants, computed for the study location (Athens, GA, 34°N latitude), agreed with those observed in the sunlight experiments described above. Rate constants were also computed for various times of day at 40°N latitude. The near-surface phototransformation rate constant of HEX at this latitude on cloudless days (averaged over both light and dark periods for a year) was 3.9 $hr^{-1}$, which corresponds to a half-life of 10.7 minutes (Zepp et al., 1979; Wolfe et al., 1982).

These researchers suggested that the primary phototransformation product was the hydrated form of tetrachlorocyclopentadienone ($C_5Cl_4O$, TCPD), although it was not isolated. Several chlorinated photoproducts with higher molecular weights than HEX were detected by GC/MS analysis of the reaction mixture. Photolysis of HEX in methanol gave a product identified as the dimethyl ketal of TCPD (Wolfe et al., 1982). According to Zepp et al. (1979), it is likely that TCPD exists predominantly in its hydrated form in the aquatic environment. The compound was not isolated, supposedly because it rapidly dimerizes or reacts to form higher molecular weight products.

To the contrary, other research indicates that formation of higher molecular weight products is a relatively minor pathway of phototransformation. Yu and Atallah (1977b) found that at a concentration of 2.2 mg/ℓ in water, uniformly labeled $^{14}$C-HEX was rapidly converted to water-soluble products upon irradiation with light from a mercury-vapor lamp (light energy 40-48% ultraviolet, 40-43% visible, remainder infrared). In exposures of 0.5-5.0 hours, 46-53% of the radiolabel was recovered as water-soluble products (compared with 7% at initiation), whereas the amount recovered by organic (petroleum ether) extraction decreased with increasing exposure duration from 25 to 6% (compared with 66% at initiation). No HEX was detected among the photoproducts in the organic extraction.

Butz et al. (1982) also found that $^{14}$C-HEX, when dissolved and irradiated as above, was rapidly photodegraded. Failure to detect HEX after 10 minutes, with a detection limit of 0.13% of the starting amount, suggested a photolytic half-life under these conditions of <1.03 minutes, assuming first-order kinetics. Reaction products were extracted and radioassayed. After both 5- and 10-minute exposures, 44% of the recovered radioactivity was in the water-soluble fraction (total percent recovery was not specified). Photoproducts were purified by thin-layer chromatography (TLC) and identified by GC/MS. The authors concluded that pentachlorocyclopentenone

($C_5HCl_5O$) was the major degradation intermediate, which subsequently degraded to water-soluble products. Dimerization of pentachlorocyclopentenone to hexachloroindenone ($C_9Cl_6O$) was thought to occur by hydrolysis, rather than phototransformation, and to represent a minor pathway (Figure 5-1). Other high molecular weight compounds identified were believed to be artifacts of the GC/MS analysis of pentachlorocyclopentenone. The researchers analyzed for mirex and kepone, but did not detect either after 5 hours irradiation (Butz et al., 1982).

The environmental fate and transport of HEX was modeled in four simulated aquatic ecosystems using the Exposure Analysis Modeling System for Toxic Organic Pollutants in Aquatic Ecosystems (EXAMS) with experimentally derived constants (Table 5-1) (Zepp et al., 1979; Wolfe et al., 1982). The four ecosystems considered in the model included a 35 km x 100 m river segment; a small eutrophic pond with a 31-day retention time in the water column; and two lakes (35 ha), one eutrophic and the other a stratified oligotrophic lake. Results indicate that in the river, export from the system and photolysis were the dominant processes (Table 5-2). In the simulated pond and both lake environments, photolysis was predicted to be the dominant process, accounting for more than 80% of the HEX transformation occurring at each of these sites. Although HEX is quite reactive, the recovery times (i.e., the times needed to reduce steady-state concentration by five half-lives) in the pond and lakes were predicted to be on the order of 2-3 months. This was attributed to slow release of HEX from the bottom sediments where the photolytic rate is retarded (Wolfe et al., 1982).

5.1.2.2. **HYDROLYSIS** -- Studies of the hydrolysis of HEX indicate that at 25-30°C and in the environmental pH range of 5-9, a hydrolytic half-life of ~3-11 days is observed (Wolfe et al., 1982; Yu and Atallah, 1977a).

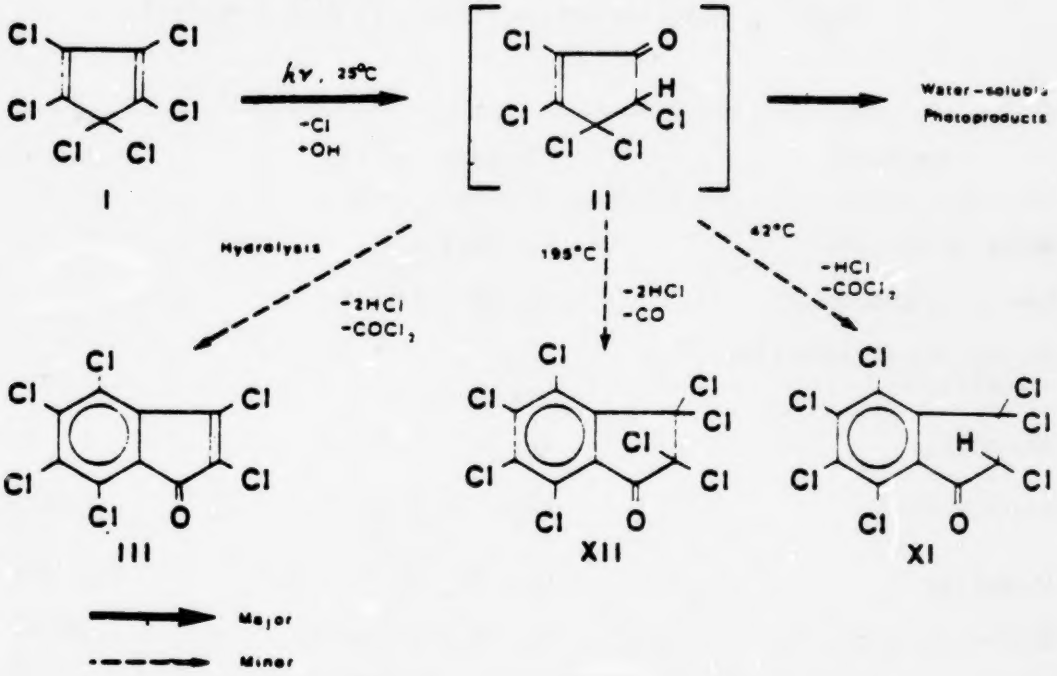

hν, 25°C
-Cl
+OH
Water-soluble Photoproducts
Hydrolysis
-2HCl
-COCl$_2$
195°C
-2HCl
-CO
42°C
-HCl
-COCl$_2$
I
II
III
XII
XI
Major
Minor

FIGURE 5-1

Proposed Pathway of Aqueous HEX Phototransformation

Source: Butz et al., 1982

## TABLE 5-1

### Summary of Constants Used in the Exposure Analysis Modeling System (EXAMS) at 25°C in Water[a]

| Constants | Symbols, Units | Values Used |
|---|---|---|
| Water solubility | $K_S$, mg/ℓ | 1.8 |
| Henry's law constant | $K_H$, atm m³/mole | $2.7 \times 10^{-2}$ |
| Octanol/water partition coefficient | $K_{ow}$ | $1.1 \times 10^{5}$ |
| Photolysis | $K_p$, $hr^{-1}$ | 3.9 |
| Hydrolysis | $K_{H_2O}$, $hr^{-1}$ | $4.0 \times 10^{-3}$[b] |
| Oxidation | $K_{ox}$, $M^{-1}$ $sec^{-1}$ | $1 \times 10^{-10}$[c] |
| Biodegradation | $K_B$, mℓ $org^{-1}$ $hr^{-1}$ | $1 \times 10^{-5}$[d] |

[a] Adapted from Wolfe et al., 1982

[b] Extrapolated to 25°C

[c] Estimated value (see Wolfe et al., 1982)

[d] This is a maximum value based on the observation that there was no detectable difference in the hydrolysis rate in either sterile or nonsterile studies and measured organism numbers (plate counts).

## TABLE 5-2

### Summary of Results of Computer Simulation of the Fate and Transport of Hexachlorocyclopentadiene in Four Typical Aquatic Environments[a]

| | River | Pond | Eutrophic Lake | Oligotrophic Lake |
|---|---|---|---|---|
| Accumulation factor | $5.4x10^4$ | 2.4 | 17 | 54 |
| Distribution (percent) | | | | |
| Water column | 1.22 | 14 | 12.97 | 2.91[b] |
| Sediment | 98.78 | 86 | 87.03 | 97.09 |
| Recovery time[c] (days) | 52 | 81 | 58 | 87 |
| Load reduction (percent) by processes: | | | | |
| Hydrolysis | 8.04 | 17.85 | 8.29 | 16.50 |
| Oxidation | 0.00 | 0.00 | 0.00 | 0.00 |
| Photolysis | 18.68 | 80.39 | 89.18 | 82.41 |
| Biodegradation (biolysis) | 0.57 | 0.23 | 0.30 | 0.01 |
| Volatilization | 0.69 | 1.33 | 1.56 | 1.08 |
| Export[d] | 72.02 | 0.20 | 0.01 | 0.00 |

[a] Adapted from Wolfe et al., 1982, with correction applied.

[b] Value was incorrectly reported as 32.91 in original paper.

[c] The time needed to reduce steady-state concentration by five half-lives.

[d] Physical loss from the system by any pathway other than volatilization.

Hydrolysis is much slower than photolysis (see Table 5-1), but may be a significant load-reducing process in waters where photolysis and physical transport processes are not important (i.e., in deep, non-flowing waters).

Wolfe et al. (1982) found hydrolysis of HEX to be independent of pH over a range of 3-10. The rate was adequately described by a neutral hydrolysis rate constant ($K_{H_2O}$ ± standard deviation) of $(1.5 \pm 0.6) \times 10^{-6}$ $sec^{-1}$ at 30°C, which corresponds to a half-life of 5.35 days. The rate constant was dependent on temperature at pH 7.0 with the half-life estimated to be 3.31, 1.71 and 0.64 days at 30, 40 and 50°C, respectively. The addition of various buffers or 0.5 M NaCl did not affect the hydrolysis rate constant, suggesting that the rate constant obtained would be applicable to marine environments as well. The addition of natural sediments sufficient to adsorb up to 92% of the compound caused the rate constant to vary by less than a factor of 2. It was therefore concluded that sorption to sediments would not significantly affect the rate of hydrolysis (Wolfe et al., 1982).

Some variability of hydrolysis rate with changes in pH was demonstrated by Yu and Atallah (1977a). They studied the stability of $^{14}C$-HEX in water at pH 3, 6, 9 and 12 at 25°C and 45°C, under dark conditions. HEX was relatively unstable at alkaline pH. At 25°C, the half-lives were 11.4, 11.4 and 6.0 days at pH 3, 6 and 9, respectively, and <2 hours (0.1 day) at pH 12. At 45°C the half-lives at pH 3, 6 and 9 were 9.2, 10.6 and 4.4 days, respectively. Degradation of HEX resulted in water-soluble products, and based upon their chromatographic behavior, the hydrolysis products appear to be polyhydroxy compounds, with $CO_2$ as a minor hydrolysis product.

In the Wolfe et al. study (1982), a preliminary investigation was conducted to determine the products from hydrolysis. The hydrolysis reaction was conducted at 60-70°C in 40% acetonitrile-water at $10^{-4}$ M HEX and proceeded through approximately two half-lives. After extraction and concentration of the lipophilic reaction products, analysis by GC/MS showed nine major peaks in the chromatogram. Several of these were high molecular weight compounds, but, as with the Yu and Atallah study (1977a), identification was not positive.

The degradation of HEX by hydrolysis in the EXAMS model environments, consisting of a simulated river, pond, eutrophic lake and oligotrophic lake were estimated to be 8.0, 17.9, 8.3 and 16.5%, respectively, of the total initially present (see Table 5-2). Hydrolysis in these aquatic environments was considered to be minor relative to photolysis in the overall degradation of HEX (Wolfe et al., 1982). The above data indicate that at neutral pH the hydrolysis half-life is from 3-11 days, compared with a much more rapid photolytic half-life of <10 minutes.

5.1.2.3. **OXIDATION** -- HEX is not expected to be oxidized under ordinary environmental conditions. In the laboratory, HEX has been reported to react with molecular oxygen at 95-105°C to form a mixture of hexachlorocyclopentenones (Molotsky and Ballweber, 1957). However, based on an estimated first order oxidation rate constant of $1 \times 10^{-10}$ $M^{-1}$ $sec^{-1}$ at 25°C in water (see Table 5-1), the EXAMS computer simulation of Wolfe et al. (1982) predicted that HEX would not be oxidized in the simulated river, pond, eutrophic lake or oligotrophic lake (see Table 5-2).

5.1.2.4. **BIODEGRADATION** -- Tabak et al. (1981) stated that HEX is biodegraded fairly rapidly in a static laboratory culture. Bottles containing HEX added to 5 mg yeast extract/ℓ as the synthetic medium were inoculated with an unspecified domestic wastewater and kept in the dark at 25°C.

Extractions for GC were done with 20 mℓ portions of methylene chloride (neutral pH) at an efficiency of >75%. HEX at 5 and 10 mg/ℓ (concentrations exceeding the compound's aqueous solubility limits) was degraded below the GC method minimum sensitivity limits (0.1 mg/ℓ) in 7 days. Volatilization was stated not to occur during a 10-day period in which control bottles having no inoculum were observed. The importance of chemical hydrolysis was not discussed by the authors. According to studies presented in Section 5.1.2.2., 7 days could represent as much as 1-2 hydrolytic half-lives, accounting for loss by as much as a factor of 4. Based on nominal concentrations, loss of HEX in these tests exceeded a factor of 50-100; therefore, hydrolysis cannot fully account for its disappearance.

Atallah et al. (1980) reported an aqueous aerobic biodegradability study to determine if, and at what rate, HEX can be degraded to $CO_2$. The inoculum was a mixed acclimated culture containing secondary municipal waste effluent and several strains of Pseudomonas putida. HEX, labeled with $^{14}C$, was the sole source of carbon in the study, with the exception of trace levels of vitamins. Total removal of $^{14}C$, primarily as volatile organics, was >80% in the first day in both uninoculated (45 mg/ℓ HEX) and inoculated (4.5 and 45 mg/ℓ HEX) media, although removal was slightly higher in inoculated media. $^{14}CO_2$ was released from both media, indicating $CO_2$ was a product from hydrolysis as well as biodegradation. The rate of conversion to $CO_2$ was initially higher in the uninoculated, but after a week, became higher in the inoculated media (Figure 5-2).

These studies show clearly that HEX can be biodegraded in aquatic media under laboratory conditions. However, Wolfe et al. (1982) stated that they failed to detect any difference in the HEX degradation rate between hydrolysis experiments where sterile and nonsterile natural sediments were added

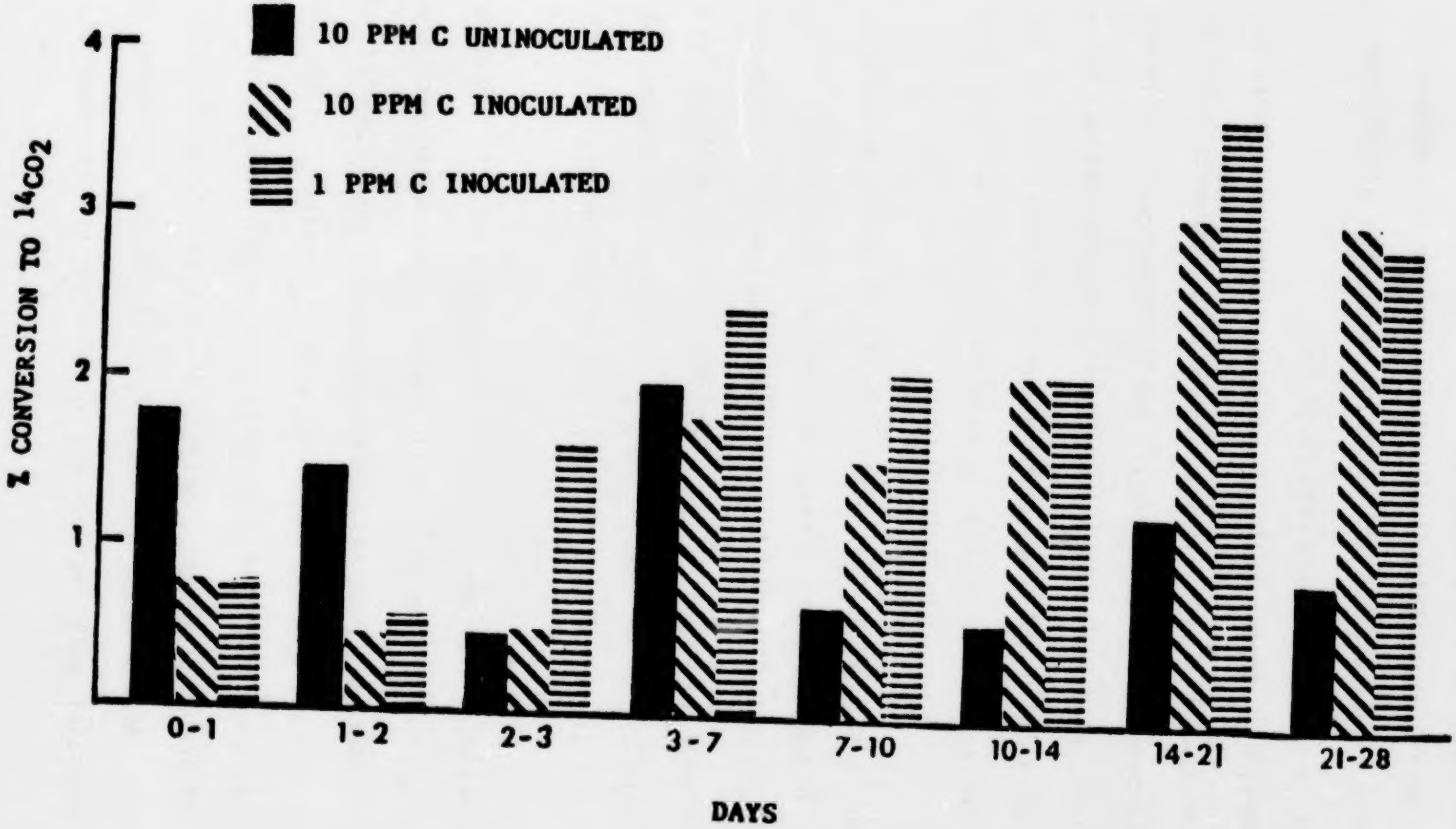


FIGURE 5-2

Rate of Degradation of $^{14}$C-HEX to $^{14}CO_2$

Source: Atallah et al., 1980

to water (1.0 g/100 mℓ). Thus they calculated a relatively low value (1x10$^{-5}$ mℓ org$^{-1}$ hr$^{-1}$; see Table 5-1) as a maximum biodegradation rate, and consequently biodegradation was estimated to be a relatively unimportant fate process in the EXAMS model (see Table 5-2).

5.1.2.5. **ADSORPTION** -- On the basis of computer simulations, Wolfe et al. (1982) predicted that HEX would adsorb strongly to sediments found in various aquatic environments (see Table 5-2). The distribution in sediments from a simulated river, pond, eutrophic lake and oligotrophic lake was estimated to be 98.8, 86.0, 87.0 and 97.1%, respectively, of total HEX in the system. The sorptive properties of HEX in relation to soil are discussed below.

5.1.3. Soil. Upon release onto soil, HEX is likely to adsorb strongly to any organic matter or humus present (Kenaga and Goring, 1980; Weber, 1979). With time, HEX concentrations should decrease as populations of soil microorganisms better adapted to metabolize HEX increase (Rieck, 1977b,c; Thuma et al., 1978). Volatilization (See Section 5.2.3.), photolysis, and various chemical processes may also dissipate the compound in certain soil environments.

5.1.3.1. **ADSORPTION** -- The soil adsorption properties of compounds such as HEX can be predicted from their soil organic carbon-water partition coefficients ($K_{oc}$). Kenaga (1980) examined the adsorption properties of 100 chemicals and concluded that compounds with $K_{oc}$ values >1000 are tightly bound to soil components and are immobile in soils. Those possessing values below 100 are adsorbed less strongly and are considered moderately to highly mobile. Accordingly, the theoretical $K_{oc}$ value is useful as an indicator of potential soil leachability or binding of the chemical.

The $K_{oc}$ values also indicate whether a chemical is likely to enter water by leaching or by being adsorbed to eroded soil particles. Because $K_{oc}$ values for HEX are not available in the literature, these values were calculated using the mathematical equation developed by Kenaga and Goring (1980) and Kenaga (1980). The equation used was:

$$\log K_{oc} = 3.64 - 0.55 (\log WS)$$

where WS is water solubility (mg/ℓ), and the 95% confidence limits are ±1.23 orders of magnitude (OM). The calculated range of $K_{oc}$ values for HEX using the reported water solubility values of 2.1 mg/ℓ (Dal Monte and Yu, 1977), 1.8 mg/ℓ (Wolfe et al., 1982) and 0.805 mg/ℓ (Lu et al., 1975) are 2903, 3159 and 4918, respectively. These calculated $K_{oc}$ values are all >1000, suggesting that HEX is tightly bound to soil components and immobile in the soil compartment. Similarly, Briggs (1973) concluded that compounds with a log octanol/water partition coefficient (log P) >3.78 are immobile in soil. The measured log P value of HEX is 5.04 (Wolfe et al., 1982), further indicating that the compound is immobile with respect to leaching.

The only sorption data found in the literature were for an experimentally flooded soil. Weber (1979) reported that an average of 68% of applied HEX was adsorbed to Cape Fear loam soil present in aqueous solutions. In these experiments, aqueous solutions (50 mℓ) containing 0.0, 0.41 (1.5 μM), 0.82 (3.0 μM) and 1.64 (6.0 μM) mg/kg of $^{14}$C-HEX were added to soil samples (0.50 mg) in stoppered bottles, which were shaken at room temperature for 24 hours. Standards, controls and two replications were used in all cases. The difference between the initial and the 24-hour equilibrium concentration of radiolabel in water was considered to be the amount of HEX adsorbed to soil. Less than 5% of the radiolabel was lost from the bottles over the 24-hour period. About 62, 66 and 75% of the applied dose was adsorbed to the soil at 0.41, 0.82 and 1.64 ppm concentrations, respectively. Weber (1979) suggested that the HEX is very strongly adsorbed by organic soil colloids because of its lipophilic character.

**5.1.3.2. BIODEGRADATION** -- The metabolism of HEX by soil microorganisms apparently is an important process in its environmental degradation. Soil degradation is rapid under nonsterile aerobic and anaerobic conditions, with indirect evidence for microbial involvement reported by Rieck (1977b,c). In one of his studies, Rieck (1977b) used several types of treatments and soil pHs to determine if the biodegradation of HEX in Maury silt loam soil was either biologically or chemically mediated, or both (Figure 5-3). Soils were incubated in glass flasks covered with perforated aluminum foil and kept on a laboratory shelf, presumably exposed to ambient lighting through the flask walls. When $^{14}$C-HEX was applied to nonsterile soil at 1 mg/kg, only 6.1% was recovered as nonpolar material (either HEX or nonpolar degradation products) 7 days after treatment, and ~71.7% was polar and unextractable material. Adjustment of pH to 4 or 8 had little effect on these results. By comparison, in autoclaved soil (the control), 36.1% of the applied dose was recovered as nonpolar material and only 33.4% recovered as polar and unextractable material (see Figure 5-3). The degradation of HEX under anaerobic (flooded) conditions occurred at a slightly faster rate than under aerobic conditions. However, no sterile, flooded control was used to determine the effects of hydrolysis, which could have accounted for the observed difference in this treatment. The mean total recovery in all treatments decreased from 67% at 7 days to 55% at 56 days. This decrease was attributed to volatilization of HEX and/or its degradation products.

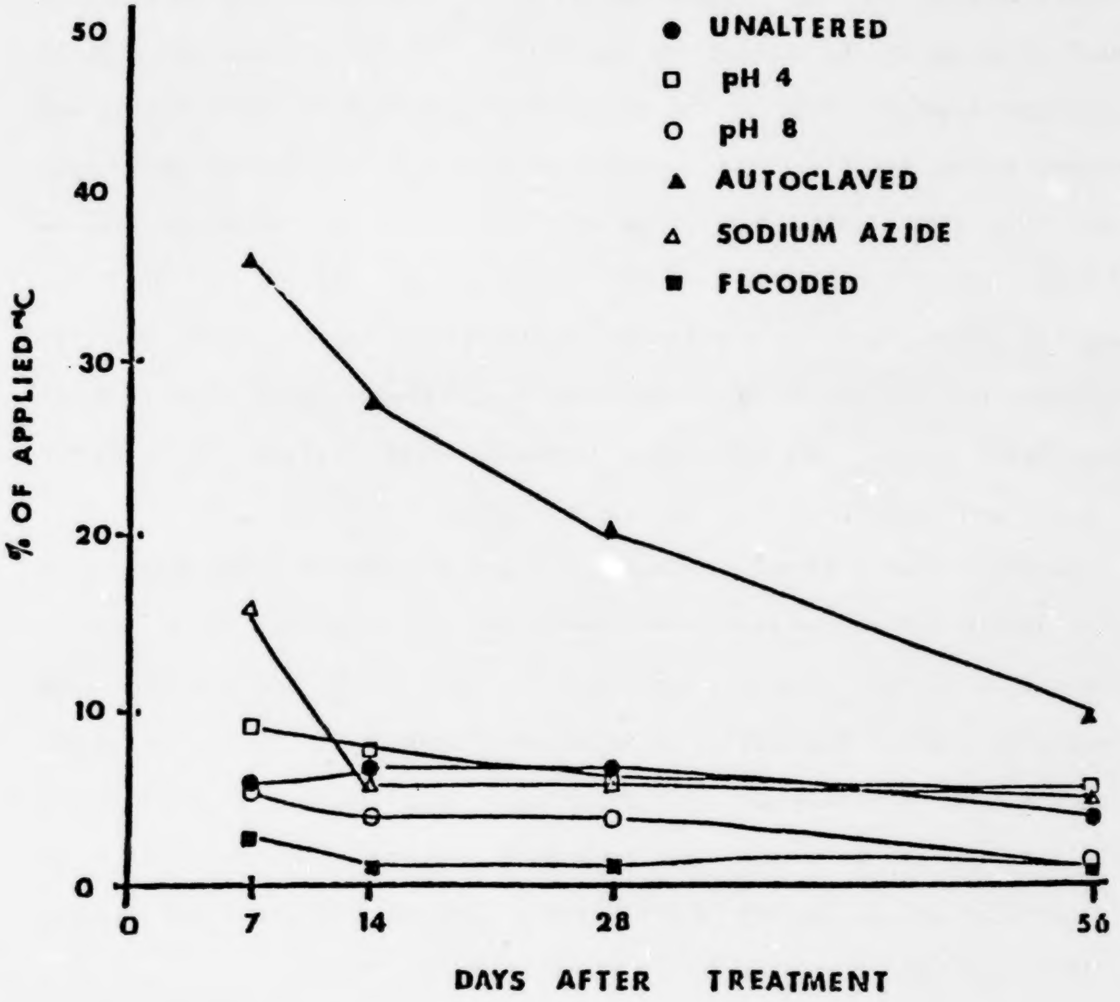


FIGURE 5-3

Persistence of Nonpolar $^{14}C$ when $^{14}C$-HEX is Applied to Unaltered and Altered Soils

Source: Adapted from Rieck, 1977b

Volatilization from soil was examined in another experiment (Rieck, 1977c). In a 14-day study, radiocarbon volatilized from nonsterile, $^{14}$C-HEX-treated soil was trapped and assayed. Over the study duration, a total of 20.2% of the applied $^{14}$C was trapped; 11.2% in hexane and 9.0% in ethanolamine-water. Most of the hexane fraction (9.3% of applied $^{14}$C) was trapped during the first day, probably representing volatilized HEX. However, the ethanolamine-water fraction, considered to represent evolved $CO_2$, was released gradually over the 14-day period. In the soil analysis, nonpolar (extractable) and polar (extractable and unextractable) material accounted for 3.4 and 40.0% of the dose, respectively, during the 14 days; thus, total recovery was only 63.6% including volatilization. No metabolic products were identified in either study by Rieck (1977b,c).

In these studies (Rieck, 1977b,c), HEX was degraded to polar material in both sterile and nonsterile soils, indicating the occurrence of an abiotic degradation process such as hydrolysis by soil water and possibly some photolysis. Since degradation occurred more quickly in nonsterile soils, biodegradation evidently was also occurring. Volatilization of HEX occurred mainly during the first day, and apparently represented no more than 11.2% of the total amount applied (Rieck, 1977c), although the low total recovery in this experiment decreases the reliability of this figure.

Under contract with the U.S. EPA, Thuma et al. (1978) studied the feasibility of using selected pure cultures (organisms not identified) to biodegrade spills of hazardous chemicals on soils, including HEX. They tested 23 organisms and found that from 2-76% of the HEX had been removed from the aqueous culture medium within 14 days. Seven of the 23 organisms degraded more than 33% of the HEX within 14 days (Table 5-3). Losses of HEX, other than biodegradation, were accounted for by the use of controls.

## TABLE 5-3

### Microbial Degradation of HEX During 14-day Exposure in a Test Medium*

| Organism Code Number | HEX Remaining in Test Medium (ppm) | Percent Degraded Relative to Control |
|---|---|---|
| Control 1 | 635 | -- |
| Control 2 | 630 | -- |
| 006 | 410 | 35 |
| 016 | 415 | 34 |
| 020 | 410 | 35 |
| 022 | 150 | 76 |
| 123 | 395 | 38 |
| 369 | 350 | 45 |
| 505 | 265 | 58 |

*Source: Adapted from Thuma et al., 1978

These studies indicate that the persistence of HEX in soil is brief, with degradation of >90% of applied HEX to nonpolar products within ~7 days. Factors contributing to this loss include abiotic and biotic degradation processes and volatilization, although the relative importance of each is difficult to quantify given the limited information available.

## 5.2. TRANSPORT

5.2.1. **Air.** The vapor pressure, water solubility, vapor density, adsorption properties, rapid photolysis (Wolfe et al., 1982) and high reactivity (Callahan et al., 1979) of HEX combine to affect its atmospheric transport. The atmospheric transport of HEX vapor from a closed waste site at Montague, MI was demonstrated by Peters et al. (1981). At an unspecified distance downwind of the site, HEX was detected in air at concentrations of 0.032-0.053 ppb (0.36-0.59 $\mu g/m^3$). Based on the concentration ratio of HEX and a tracer gas released at a known rate, the average HEX emission rate during the measurement period was calculated to be 0.26 g/hour

Volatilization of HEX from water may occur following either industrial discharge [e.g., a concentration of 18 mg/ℓ was found in the aqueous discharge at a Memphis pesticide plant (U.S. EPA, 1980c)] or accidental spill. The tendency of HEX to adsorb to organic matter in water or soil would limit the compound's volatility, as would suspended solids in surface water. Transport of HEX vapor will also be limited by the estimated atmospheric residence time, based on photolysis, hydrolysis and ozone reaction rates, of ~5 hours (Cupitt, 1980). HEX adsorbed onto aquatic or terrestrial particles may also enter the atmosphere and be transported in the air for a time while being transformed by photolysis or other processes.

As part of an experiment with chlordane, Bevenue and Yeo (1969) found some interesting vaporization and adsorption properties of HEX, which may exist in an amount as large as 1% in commercially available chlordane. Small quantities of HEX (0.5 mg) in open vials were placed in closed glass vessels containing 20 mℓ of either distilled water or isooctane, so that only vaporized HEX could contact the solvent. Vessels were stored under fluorescent lighting. Gas chromatographic data from the solutions of distilled water initially revealed the presence of adsorbed vapor of HEX and its degradation products, indicating transport from air to water. Beyond 3 days exposure, however, the chemical and its products had completely disappeared from the GC chromatogram, indicating either dissipation or decomposition of the compound. The data obtained from the isooctane solutions revealed a different GC pattern. No degradation was observed after 24 hours, while a multiple-peak chromatogram (indicating degradation products) was obtained after the solutions were exposed 7-21 days. This chromatogram suggests that the compound may be susceptible to atmospheric oxidation and photodecomposition or both (NCI, 1977). The more rapid disappearance of compound and degradation products in water than in the isooctane solution may further indicate the occurrence of hydrolysis.

More information on the volatility and adsorption of HEX is presented in Sections 5.2.2. and 5.2.3., respectively.

5.2.2. **Water.** HEX introduced into water bodies may be transported in either undissolved, dissolved or adsorbed forms. In its undissolved form, HEX will tend to sink because of its high specific gravity and may then become concentrated in deeper waters, where photolysis and volatilization would be precluded. Some HEX may be dissolved in water (up to ~2 mg/ℓ) and then be dispersed with water flow (i.e., in a river). HEX tends to adsorb onto organic matter because of its lipophilic nature and may then be transported with water flow in a suspended form. Transport to the air may occur by volatilization, which was measured in laboratory studies (Kilzer et al., 1979; Weber, 1979) and predicted using the EXAMS model by Wolfe et al. (1982). However, suspended solids in surface water may be a major factor in reducing volatilization.

Weber (1979) measured the volatility of $^{14}$C-HEX from distilled water following the incubation of the glass-stoppered and unstoppered test bottles shaken at room temperature for 24 hours. Experiments were performed with standard HEX solutions of $1.5 \times 10^{-6}$ M (0.41 mg/ℓ) in distilled water, with readings taken 24 hours later. From the full glass-stoppered bottles, only 4-5% of the HEX was lost, while in the half-full stoppered bottles, 15-16% of the chemical was missing. This suggests that head space in the bottle contributed to the loss of HEX. The volatility of HEX was shown by the loss of 45-47% from the half-full, unstoppered bottles over the 24-hour period.

Kilzer et al. (1979) determined the rate of $^{14}$C-HEX volatilization from water as a function of the rate of water evaporation. Bottles containing aqueous HEX solutions (50 μg/ℓ) were kept without shaking at 25°C. The escaping vapor condensed on a "cold finger" and was quantified by liquid scintillation spectroscopy. Based on recovery of added label, the HEX volatilization rates for the first and second hours of testing were calculated to be 5.87 and 0.75%/mℓ $H_2O$, respectively. Since the water evaporation rate was 0.8-1.5 mℓ/hour, rates for HEX were within the ranges of 4.7-8.8 and 0.6-1.1%/hour, respectively. These results suggest that a fairly rapid initial volatilization occurred at the water surface, and that by the second hour, diffusion of HEX to the water surface may have been limiting because of the static conditions of the test. If the rate observed during the second hour had continued for the remainder of 24 hours, total loss would have been ~18-34%, or somewhat less than that observed in the test by Weber (1979) where unstoppered bottles were shaken.

In the aqueous biodegradation test of Atallah et al. (1980) described in Section 5.1.2.4., a very high rate of volatilization was determined. Over 80% of the radiolabel added as $^{14}$C-HEX had disappeared after the first day, even from uninoculated media. Most was found to have volatilized (total recovery averaged 94%) and was primarily in organic form. The physical conditions of the test, such as covering, shaking or aeration of test solutions, were unspecified. In addition, disappearance of label at initiation was >50%. This peculiarity was not explained, but could be due to the use of HEX concentrations of 4.5 and 45.3 mg/ℓ, which exceed the limit of aqueous solubility.

Wolfe et al. (1982) also studied the evaporation rate of HEX from water and experimentally determined the Henry's law constant (H) to be 0.027±0.010 atm m$^3$/mole. This value corresponds with 0.0137 and 0.0357 atm m$^3$/mole calculated from the measured vapor pressure (0.08 mm Hg at 25°C) (Irish, 1963) and the water solubilities (2.1 and 0.805 mg/ℓ) (Dal Monte and Yu, 1977; Lu et al., 1975, respectively), according to the following equation:

$$H = \frac{\text{Vapor pressure (atm)}}{\text{Water solubility (mole/m}^3\text{)}}$$

The mathematical EXAMS model (see Section 5.1.2.) was used to indicate the relative importance of volatilization and other processes in the fate and transport of HEX (load reduction) of four aquatic systems (see Table 5-2). The model indicated that volatilization of HEX from a river, pond, eutrophic lake and oligotrophic lake would account for only 0.69, 1.33, 1.56 and 1.08% of load reduction, respectively. These values are quite low compared with the laboratory values described previously. This discrepancy is apparently due to the fact that the model estimates that 86-99% of the HEX present in these systems will be adsorbed to sediment, and thus will not be subject to volatilization. Experiments measuring vaporization of HEX from water-sediment systems have not been conducted.

Export (i.e., physical loss by methods other than volatilization) was predicted to be a very important transport mechanism in the simulated river environment (Wolfe et al., 1982). Using the EXAMS model, export accounted for load reductions of 72% in the river, as compared with the three nonflowing environments mentioned previously, where photolysis was the dominant removal mechanism.

5.2.3. Soil. As indicated previously (Section 5.1.3.1.), HEX in soils is predicted to be tightly adsorbed to organic matter and relatively resistant to leaching by soil water. Thus, the primary routes of transport for soil applied HEX are by movement of particles to which it is adsorbed or by volatilization. No data are available pertaining to HEX transport on soil particles; however, a few studies have determined the rate of volatilization from soils.

Kilzer et al. (1979) determined that $^{14}$C-HEX volatilized from moist soils (sand, loam and humus) at a faster rate in the first hour than in the second hour of the study. HEX (50 μg/kg) was placed in bottles with each soil type and shaken vigorously. The bottles were incubated for 2 hours at 25°C, apparently without shaking. The evaporating HEX condensed on a "cold finger" and was quantified by liquid scintillation counting. For sand, loam and humus, the volatilization rate was expressed as the percentages of applied radioactivity per mℓ of evaporated water and were for the first hour 0.83, 0.33 and 0.14%, while for the second hour they were 0.23, 0.11 and 0.05%, respectively. Volatilization was much higher from the sand.

For HEX and nine other tested chemicals, Kilzer and coworkers found that the volatilization rate from distilled water cannot be used to predict the rate from wetted soils. Among the chemicals tested, there was no correlation between water solubility or vapor pressure and volatilzation from soils. The volatilization rate for HEX in soil was primarily dependent upon soil organic matter content, mainly because of the highly adsorptive properties of HEX.

Rieck (1977c) measured the rate of volatilization of HEX from Maury silt loam soils (see Section 5.1.3.2.). Following the application of 100 mg of $^{14}$C-HEX to soil, the cumulative evaporation of HEX and its nonpolar metabolites (penta- and tetrachlorocyclopentadiene) on days 1, 2, 3, 5, 7 and 14 were 9.3, 10.2, 10.6, 10.8, 11.0 and 11.2%, respectively. The results indicate that HEX evaporation to air occurred mainly during the first day following application and was probably associated with the surface soil only.

When compared with data presented in the preceding section (5.2.2.), these studies demonstrate that HEX volatilizes from soils much more slowly than from sediment-free water. This difference is most likely due to adsorption of HEX to the soil matrix, and possibly to slow diffusion to the soil surface.

## 5.3. BIOCONCENTRATION/BIOACCUMULATION

The occurrence of toxic substances in the environment raises the issues of whether humans may be exposed to them by air, water or food and, if so, what are the physiological exposures? The transport and fate of HEX (see Sections 5.1. and 5.2.) are the primary determinants of human exposure to the environmental sources of these compounds, but the more crucial physiological exposure levels are determined by the manner in which a compound crosses biological membranes. Bioaccumulation, alternately sometimes expressed as biological persistence, is the net result of the absorption and elimination rate of a compound and, therefore, determines the level and duration of human physiological exposure.

The terminology used in this section will follow that of Macek et al. (1979): bioconcentration implies that tissue residues result only from exposure to the ambient environment (e.g., air for terrestrial or water for aquatic species); bioaccumulation considers all exposures (air, water and food) of an individual organism as the source of tissue residues; and biomagnification defines the increase in tissue residues observed at successively higher trophic levels of a food web.

The log octanol/water partition coefficient (log P) of HEX has been experimentally determined to be 5.04 (Wolfe et al., 1982) and 5.51 (Veith et al., 1979), which would indicate a substantial potential for bioconcentration, bioaccumulation and biomagnification. Actual determinations of bioconcentration and bioaccumulation in several aquatic organisms, however, indicate that HEX does not accumulate to a great extent (Podowski and Khan, 1979, 1984; Veith et al., 1979; Spehar et al., 1979; Lu et al., 1975), mainly because it is metabolized rapidly.

Podowski and Khan (1979, 1984) conducted several experiments concerning the uptake, bioaccumulation and elimination of $^{14}$C-HEX in goldfish (Carassius auratus) and concluded that the species eliminated absorbed HEX rapidly. In one experiment, fish were transferred daily into fresh solutions of $^{14}$C-HEX for 16 days. This transfer of three fish/jar resulted in accumulative exposure of 240 μg of HEX. Nominal HEX concentrations of 10 μg/ℓ resulted in measured water concentrations (based on radioactivity) in the range of 3.4-4.8 μg/ℓ, because of rapid volatilization of the compound. Radioactivity accumulated rapidly in fish tissue, reaching a maximum on day 8 corresponding to ~6 mg HEX/kg. Since an undetermined amount of the radioactivity was present as metabolites, no bioconcentration factor can be calculated. From day 8 to day 16, tissue levels declined in spite of daily renewal of exposure solutions, indicating that excretion of HEX and/or its metabolites was occurring more rapidly than uptake. In a static exposure to an initial measured HEX concentration of 5 μg/ℓ, radioactivity was taken up by the fish to a level corresponding to 1.6 mg HEX/kg on day 2, accompanied by a slight decrease of HEX in the water. By day 4, ~50% of the absorbed activity had been excreted, and the water level increased. Over the following 12 days, radioactivity in both water and fish declined slowly.

Podowski and Khan (1979, 1984) also studied elimination, metabolism and tissue distribution of HEX injected intraperitoneally into goldfish and concluded that goldfish eliminate injected HEX both rapidly and linearly (biological half-life ~9 days). Fish (27-45 g) were injected with 39.6 μg of $^{14}$C-HEX and analyzed 3 days later. Of the 97% of the radiolabeled dose accounted for, ~18.9% was eliminated by the fish, leaving a residual of 78.1%. Of the residue found in the fish, 47.2% was extractable in organic solvent (little of the radiolabeled material could be identified as HEX, which indicated that biotransformation had occurred); 10.6% was water soluble metabolites; and 20.3% was unextractable. None of the metabolites were identified. A biphasic elimination was observed -- rapid at first, followed by a slower phase.

In another part of these studies, residual activity in several fish tissues was assayed 2, 4, 6 and 8 days following an injection of 38.4 μg/fish of $^{14}$C-HEX. Results showed activity corresponding to 0.2 and 0.3 mg HEX/kg in the spinal cord and gills, respectively. These concentrations were constant throughout the 8-day period of the study. Residues in the kidneys and bile increased within the same period from 1-3 and 0-32 mg/kg, respectively, indicating elimination by these routes. The authors stated that the increase was probably from enhanced conversion of the parent compound into polar products suitable for elimination. In the other tissues, all residual levels dropped leaving only the liver with levels >1 mg/kg (Podowski and Khan, 1979, 1984). The authors did not identify the metabolites because of the complications created by the fact that HEX and its metabolites are very reactive and extremely lipophilic. When the fat was removed to purify the HEX, over 90% of the radioactivity levels initially accounted for in the goldfish were lost.

Veith et al. (1979) determined the bioconcentration factor (BCF) for HEX to be 29 in the fathead minnow (Pimephales promelas). In a 32-day flow-through study, 30 fish were exposed to HEX at a mean concentration of 20.9 μg/ℓ and were sacrificed five at a time for residue analysis at 2, 4, 8, 16, 24 and 32 days. The study was conducted using Lake Superior water at 25°C (pH 7.5, dissolved oxygen >5.0 mg/ℓ and hardness 41.5 mg/ℓ as $CaCO_3$). On the basis of its estimated octanol/water partition coefficient alone (log P = 5.51), a BCF of ~9600 would have been predicted. However, HEX did not bioconcentrate substantially, and therefore deviated from the log P:log BCF relationship shown for most of the other 29 chemicals tested by these researchers.

Spehar et al. (1979) conducted a 30-day early-life-stage, flowthrough toxicity test at 25°C with the fathead minnow (P. promelas). HEX residues in the fish after 30 days of continuous exposure to HEX were <0.1 mg/kg for all concentrations tested (0.78-9.1 μg/ℓ), and the BCF was <11 (0.1 mg/kg in fish divided by 9.1 μg/ℓ in water). In addition, toxicity results indicated that a median lethal threshold (or incipient $LC_{50}$) was attained within 4 days. The authors concluded that the rapid attainment of a threshold toxicity level and the low BCF indicate that HEX is noncumulative.

Lu et al. (1975) studied the fate of HEX in a model terrestrial-aquatic ecosystem maintained at 26.7°C with a 12-hour photoperiod. The model ecosystem consisted of 50 sorghum (Sorghum vulgare) plants (3-4 inches tall) in the terrestrial portion; 10 snails (Physa sp.), 30 water fleas (Daphnia magna), filamentous green algae (Oedogonium cardiacum) and a plankton culture were added to the aquatic portion. The sorghum plants were treated topically with 5.0 mg of $^{14}$C-HEX in acetone to simulate a terrestrial application of 1.0 lb/acre (1.1 kg/ha). Ten early-fifth-instar caterpillar larvae (Estigmene acrea) were placed on the plants. The insects consumed most of the treated plant surface within 3-4 days. The feces, leaf grass and the larvae themselves contaminated the moist sand, permitting distribution of the radiolabeled metabolites by water throughout the ecosystem. After 26 days, 300 mosquito larvae (Culex pipiens quinquefasciatus) were added to the ecosystem, and on day 30, three mosquito fish (Gambusia affinis) were added. The experiment was terminated after 33 days, and the various parameters were analyzed. The radioactivity was then extracted from water with diethyl ether and from organisms with acetone. The results of TLC analysis of the extracts are presented in Table 5-4. Data were not reported for Daphnia or the salt marsh caterpillar. Uptake in this experiment occurred through food as well as water, and therefore is termed bioaccumulation rather than bioconcentration. Lu et al. (1975) used the term ecological magnification (EM) to designate the bioaccumulation factor (BAF).

## TABLE 5-4

### Relative Distribution of HEX and Its Degradation Products[a]

| | $^{14}$C-HEX Equivalents (ppm) | | | | |
|---|---|---|---|---|---|
| | Water (μg/ℓ) | Algae (mg/kg) | Snail (mg/kg) | Mosquito Larva (mg/kg) | Fish (mg/kg) |
| HEX | 0.00024 | 0.0818 | 0.3922 | 0.2230 | 0.1076 |
| Other extractable compounds | 0.00204 | 0.1632 | 0.3824 | 0.2542 | 0.1542 |
| Total extractable $^{14}$C[b] | 0.00228 | 0.2450 | 0.7746 | 0.4772 | 0.2618 |
| Unextractable $^{14}$C | 0.00750 | 0.0094 | 0.0814 | 0.0104 | 0.0982 |
| Total $^{14}$C[b] | 0.00978 | 0.2544 | 0.8560 | 0.4876 | 0.3600 |

[a] Source: Lu et al., 1975

[b] Underlines indicate summation

The BAF for HEX in fish was 448 (0.1076 mg/kg in fish divided by 0.24 µg/ℓ in water) for the 3-day exposure period, indicating a moderate potential for concentration (Kenaga, 1980). The BAF in algae (<33-day exposure), snails (<33-day exposure) and mosquito larvae (7-day exposure) was reported to be 341, 1634 and 929, respectively (Lu et al., 1975).

Biomagnification, measured as the ratio of HEX residues between trophic levels (e.g., snail/algae or fish/mosquito), was far less substantial than bioconcentration. Based on the HEX tissue residues, the snail/algae ratio was 0.3922/0.0818 = 4.8 and the fish/mosquito ratio was 0.1076/0.2230 = 0.48.

Lu et al. (1975) also studied the metabolism of HEX by the organisms present in the model terrestrial-aquatic ecosystem. None of the products were identified except for HEX. The authors reported that unmetabolized HEX represented large percentages of the total extractable $^{14}C$, being 33% in algae, 50% in snail, 46% in mosquito and 41% in fish. Percent biodegradation was calculated for each organism [(unextractable $^{14}C$ x 100)/total $^{14}C$] and reported to be: 4% for the alga (in <33 days); 10% for the snail (in <33 days); 2% for the mosquito (in 7 days); and 27% for the fish (in 3 days). However, these values may underestimate the extent of metabolism, since acetone extractable polar compounds were not considered in the calculations.

Velsicol Chemical Corporation (1978) conducted fish tissue residue studies below their Memphis, TN facility and reported that HEX was not detected in either catfish or carp, although chlorinated compounds were detected in the fish tissue. The possible source of these other compounds was not discussed. In a joint Federal and state study of the Mississippi River above, around and below Memphis, Bennett (1982) of the U.S. EPA reported that HEX was not detected in any of the eight fish sample groups analyzed by GC/MS.

In contrast to the above-described findings, fish collected from the stream in the vicinity of the Hooker chemical plant discharge in Montague, Michigan, were reported to contain 4-18 µg/kg of HEX in the edible fillets. However, there was some question as to whether the analyzed compound was HEX or a degradation product (Swanson, 1976).

## 5.4. SUMMARY AND CONCLUSIONS

The fate and transport of HEX in the atmosphere is not known, but available information suggests that the compound does not persist. Cupitt (1980) estimated its tropospheric residence time to be ~5 hours, with photolysis and reaction with hydroxyl radicals and ozone being the key degradative processes. However, atmospheric transport of HEX from an area of stored wastes has been demonstrated, at least for a short distance (Peters et al., 1981).

In water, HEX is likely to dissipate rapidly by means of photolysis, hydrolysis and biodegradation. In shallow water (a few centimeters deep), HEX has a photolytic half-life of ~0.2 hours (Butz et al., 1982; Wolfe et al., 1982). In deeper water where photolysis is precluded, hydrolysis and biodegradation should become the key degradative processes when there is little movement from the system. The hydrolytic half-life of HEX is several days, and is not strongly affected by pH in the environmental range (5-9), by salinity or by suspended solids (Yu and Attallah, 1977a; Wolfe et al., 1982). Biodegradation may also be a significant process in certain waters (Tabak et al., 1981), although the evidence is weak. HEX is known to volatilize from water (Kilzer et al., 1979; Weber, 1979). It is probable that volatilization is limited by diffusion, that is, loss from deeper waters would occur very slowly unless vertical mixing has taken place. Sorption on sediments may also retard volatilization.

The fate and transport of HEX in soils are affected by its strong tendency to adsorb onto organic matter (Kenega and Goring, 1980; Wolfe et al., 1982; Weber, 1979). HEX is predicted to be relatively immobile in soil based on its high log P value (Briggs, 1973). Volatilization, which is likely to occur primarily at the soil surface, is inversely related to the organic matter levels and water-holding capacity of the soil (Kilzer et al., 1979). Leaching of HEX by groundwater should be very limited, and chemical hydrolysis and microbial metabolism are expected to reduce environmental levels. HEX is metabolized by a number of unidentified soil microorganisms (Rieck, 1977b,c; Thuma et al., 1978).

The bioconcentration/bioaccumulation/biomagnification potential of HEX would appear to be substantial based on its high lipophilicity. BAFs derived from a short-term model ecosystem study appear to indicate a moderate accumulation potential for algae (BAF = 341), snails (1634), mosquito larvae (929) and mosquito fish (448). However, the compound did not substantially biomagnify from algae to snails or from mosquito larvae to fish (Lu et al., 1975). In addition, steady-state bioconcentration factors (BCFs) in fish, measured in 30-day flow-through exposures to constant levels of HEX, were only 29 and <11, respectively (Veith et al., 1979; Spehar et al., 1979). Metabolism and excretion of HEX by goldfish were demonstrated by Podowski and Khan (1979).

# 6. ECOLOGICAL EFFECTS

The effects of HEX have been reported for several aquatic organisms, including invertebrates and fish from freshwater and saltwater environments and saltwater algae. The bioconcentration potential of HEX in aquatic organisms and ecosystems has also been studied; these data have been discussed in Section 5.3. The effects on microorganisms have been examined to some degree. However, few studies have been located which describe the effects of HEX on terrestrial plants or vertebrates.

## 6.1. EFFECTS ON AQUATIC ORGANISMS

### 6.1.1. Freshwater Aquatic Life.

6.1.1.1. ACUTE TOXICITY -- Several studies are available on the effects resulting from exposure of freshwater aquatic life to various concentrations of HEX.

Two studies have reported the acute toxicity of HEX in D. magna (Buccafusco and LeBlanc, 1977; Vilkas, 1977). The results are shown in Table 6-1. The 48-hour $LC_{50}$ value ranged from 39-52 µg/ℓ, and the 48-hour no-effect level ranged from 18-32 µg/ℓ. In the study by Vilkas (1977), routine water quality parameters were also analyzed. Results showed that the pH values, determined initially and after 48 hours, increased with an increase in HEX concentration.

Results from acute toxicity tests with HEX have been reported for a number of freshwater fish species (Table 6-1). The 96-hour $LC_{50}$ value for fathead minnow larvae in a flowthrough test with measured toxicant concentrations was 7 µg/ℓ (Spehar et al., 1977, 1979). Values obtained with adult fathead minnows in static tests with unmeasured toxicant concentrations ranged from 59-180 µg/ℓ (Henderson, 1956; Buccafusco and LeBlanc, 1977). Reported 96-hour values for goldfish, channel catfish and bluegills

TABLE 6-1

Acute Toxicity Data for Freshwater Species Exposed to HEX

| Species | Method[a] | $LC_{50}$ (µg/ℓ)[b] |  |  | Acute No-Effect Concentration (µg/ℓ) | Comments | Reference |
|---|---|---|---|---|---|---|---|
|  |  | 24-hour | 48-hour | 96-hour |  |  |  |
| Cladoceran<br>*Daphnia magna* | S,U | 93.0<br>(78.9-109.6) | 52.2<br>(44.8-60.9) | ND | 32 | 17°C, soft water | Vilkas, 1977 |
| Cladoceran<br>*Daphnia magna* | S,U | 130<br>(68-260) | 39<br>(30-52) | ND | 18 | 22°C, soft water | Buccafusco and LeBlanc, 1977 |
| Fathead minnow (larvae, <0.1 g)<br>*Pimephales promelas* | FT,M | NR | NR | 7.0 | 3.7 | 25°C, soft water | Spehar et al., 1977, 1979 |
| Fathead minnow (1-1.5 g)<br>*Pimephales promelas* | S,U | 115<br>93<br>75 | 110<br>78<br>59 | 104<br>78<br>59 | NR<br>NR<br>NR | Hard water, acetone soln.<br>Soft water, acetone soln.<br>Hard water, emulsion (no acetone) | Henderson, 1956 |
| Fathead minnow (0.72 g)<br>*Pimephales promelas* | S,U | 240<br>(170-320) | 210<br>(180-250) | 180<br>(160-220) | 87 | 22°C, soft water | Buccafusco and LeBlanc, 1977 |
| Goldfish<br>*Carassius auratus* | NR | NR | NR | 78 | NR | No details given | Podowski and Khan, 1977 |
| Channel catfish (2.1 g)<br>*Ictalurus punctatus* | S,U | 190<br>(140-250) | 150<br>(130-180) | 97<br>(81-120) | 56 | 22°C, soft water | Buccafusco and LeBlanc, 1977 |
| Bluegill (0.45 g)<br>*Lepomis macrochirus* | S,U | 170<br>(140-210) | 150<br>(120-180) | 130<br>(110-170) | 65 | 22°C, soft water | Buccafusco and LeBlanc, 1977 |
| Bluegill (8-13 cm)<br>*Lepomis macrochirus* | S,U | >500,000 | 30,000 | 25,000 | NR | Water aerated during test | Davis and Hardcastle, 1957 |
| Largemouth bass (8-13 cm)<br>*Micropterus salmoides* | S,U | >500,000 | 35,000 | 20,000 | NR | Water aerated during test | Davis and Hardcastle, 1957 |

[a] S = static, FT = flowthrough, U = unmeasured concentrations, M = measured concentrations

[b] Numbers in parentheses give 95% confidence interval

were also within this range (Podowski and Khan, 1979; Khan et al., 1981; Buccafusco and LeBlanc, 1977). Anomalously high values for bluegill (25,000 μg/ℓ) and largemouth bass (20,000 μg/ℓ), well above the solubility limit of 800-2100 μg/ℓ (see Section 3.2.1.), were reported by Davis and Hardcastle (1957) (see Table 6-1). These results could be high due to the failure to properly disperse the toxicant in the test water (no carrier was mentioned), and/or to volatilization of the compound, since the water was aerated during the test.

Sinhaseni et al. (1982) have recently reported biological effects of HEX in rainbow trout (Salmo gairdneri) exposed to 130 μg/ℓ HEX in a nonrecirculating flowthrough chamber. Oxygen consumption, measured polarographically, increased by 193% within 80 minutes and then gradually decreased until death in ~5 hours. Vehicle controls showed no effects after 76 hours of exposure. HEX added to normal trout mitochondria increased basal oxygen consumption. The authors concluded that HEX uncoupled oxidative phosphorylation.

Sinhaseni et al. (1983) continued their research on the respiratory effects of HEX on intact rainbow trout. Acclimated rainbow trout were exposed to 130 ppb HEX in a flow-through well water circuit which was designed to permit measurements of oxygen consumption in fish. Again, HEX increased oxygen consumption rates (186±24%), with the maximum oxygen consumption rates being nearly the same as the previous experiment (~84 minutes). The oxygen consumption decreased until death (~6.5 hours). Control trout (acetone vehicle) showed no changes. The authors reported profound respiratory stimulation and HEX appeared to uncouple oxidative phosphorylation. Sinhaseni et al. (1983) postulated that HEX intoxication in the intact animal may be due to increased oxygen consumption and impaired oxidative ATP synthesis due to the mitochondrial uncoupling action of HEX.

6.1.1.2. SUBCHRONIC/CHRONIC TOXICITY -- Spehar et al. (1977, 1979) conducted 30-day early life stage flowthrough toxicity tests with fathead minnows (_P. promelas_). Tests were performed with measured concentrations and were initiated with 1-day-old larvae. The 96-hour $LC_{50}$ value was reported in the preceding section. The 96-hour mortality data indicated a sharp toxicity threshold, such that 94% survival was observed at 3.7 µg/ℓ, 70% at 7.3 µg/ℓ, and 2% at 9.1 µg/ℓ. At the end of the 30-day exposure period, mortality was only slightly higher, with 90% survival at 3.7 µg/ℓ, 66% at 7.3 µg/ℓ, and 0% at 9.1 µg/ℓ. These results indicated that the median lethal threshold, the lowest concentration causing 50% mortality, was attained within 4 days. In addition, the HEX residues found in fathead minnows during the end of the 30-day tests were low (<0.1 µg/g) and the BCF value was reported to be <11 (Spehar et al., 1979). The authors concluded that the toxicity data and BCF values indicated that HEX was noncumulative in fish; i.e., did not bioconcentrate in fish as a result of continuous low-level exposure to HEX. The growth rate of surviving larvae, measured as both body length and weight, did not decrease significantly at any of the concentrations tested, compared with the controls. This was true even at 7.3 µg/ℓ, a level greater than the calculated $LC_{50}$ value. Based on these toxicity and growth data, Spehar et al. (1977, 1979) concluded that 3.7 µg/ℓ was the highest concentration of HEX that produced no adverse effects on fathead minnow larvae. Thus, the maximum acceptable toxicant concentration (MATC) was in the range of 3.7-7.3 µg/ℓ. No other chronic toxicity data for any freshwater species were located.

### 6.1.2. Marine and Estuarine Aquatic Life.

6.1.2.1. ACUTE TOXICITY -- Walsh (1981) reported unpublished data on the effects of HEX on four species of marine algae, derived according to the method described by Walsh and Alexander (1980). The 7-day $EC_{50}$ was calculated as the concentration causing 50% decrease in biomass compared with the control, as estimated by absorbance at 525 nm. The 7-day $EC_{50}$ values reported indicated a wide range of susceptibility between the species tested. Isochrysis galbana and Skeletonema costatum were the most susceptible species, with the average 7-day $EC_{50}$ values reported were about 3.5 and 6.6 μg/ℓ, respectively. The average value for Porphyridium cruentum was 30 μg/ℓ, while that for Dunaliella tertiolecta was 100 μg/ℓ. Other tests with S. costatum indicated that the direct, algicidal effect of HEX was less pronounced than its effect on growth. After 48 hours of exposure to HEX at 25 μg/ℓ, mortality, as indicated by staining and cell enumeration, was only 4% (Walsh, 1983).

Among marine invertebrates, the 96-hour $LC_{50}$ values for HEX ranged from 7-371 μg/ℓ (Table 6-2) (U.S. EPA, 1980a). Except where indicated, these results were from static tests with nominal concentrations of HEX. The organism exhibiting by far the highest $LC_{50}$ was the polychaete, Neanthes arenaceodentata, which is an infaunal organism living in the sediment. The two shrimp species tested were more sensitive to HEX by a factor of 10 or more.

The static $LC_{50}$ value reported by U.S. EPA (1980a) for the grass shrimp, Palaemonetes pugio, was slightly higher than that for the mysid shrimp, Mysidopsis bahia (see Table 6-2). However, the $LC_{50}$ for the mysid shrimp was considerably lower in a flow-through test than in the static test. Similarly, the $LC_{50}$ value was lower when calculated from actual

## TABLE 6-2

## Acute Toxicity Data on Marine Organisms Exposed to HEX[a]

| Species | Method[b] | 96-hour $LC_{50}$[c] (µg/ℓ) |
|---|---|---|
| Polychaete<br>*Neanthes arenaceodentata* | S,U | 371<br>(297-484) |
| Grass shrimp<br>*Palaemonetes pugio* | S,U | 42<br>(36-50) |
| Mysid shrimp<br>*Mysidopis bahia* | S,U | 32<br>(27-37) |
| Mysid shrimp<br>*Mysidopis bahia* | FI,U | 12<br>(10-13) |
| Mysid shrimp<br>*Mysidopis bahia* | FI,M | 7<br>(6-8) |
| Pinfish<br>*Lagodon rhomboides* | S,U | 48<br>(41-58) |
| Spot<br>*Leiostomus xanthurus* | S,U | 37<br>(30-42) |
| Sheepshead minnow<br>*Cyprinodon variegatus* | S,U | 45<br>(34-61) |

[a] Source: U.S. EPA, 1980a

[b] M = measured concentrations; S = static; FI = flowthrough; U = unmeasured concentrations

[c] 95% confidence interval

measurements of HEX concentrations in the test solutions (measured concentration) than when calculated according to the concentrations based on amounts originally added to test solutions (nominal concentrations).

The acute toxicity values for HEX were comparable for each of three marine fish species tested (U.S. EPA, 1980a). The static 96-hour $LC_{50}$ values based on unmeasured concentrations for spot, sheepshead minnow and pinfish varied only from 37-48 μg/ℓ (see Table 6-2).

6.1.2.2. CHRONIC TOXICITY -- In an unpublished study (U.S. EPA, 1981), groups of 40 mysid shrimp were exposed for 28 days to measured, flow-through concentrations of HEX. From the data shown in Table 6-3, measured concentrations were about one-half of nominal. Mortality occurred in all concentrations except the control, but showed no consistent dose-response relationship. Fecundity, however, was more clearly related to dose (Table 6-3).

No other data were located on the chronic toxicity of HEX to saltwater organisms.

## 6.2. EFFECTS ON OTHER ECOSYSTEMS

The effects of HEX on microorganisms in aqueous and soil systems have been tested. Many of the aqueous concentrations tested exceeded the upper limit of aqueous solubility of 0.8-2.1 mg/ℓ; these concentrations usually were achieved by use of an organic solvent. Thus the environmental significance of the results must be interpreted with caution.

Cole (1953) inoculated 10 strains of common human and animal pathogens into growth media containing various concentrations of HEX. The inhibiting concentration, or lowest concentration in which no growth was observed after 96 hours of contact, ranged from 1-10 mg/ℓ HEX. Addition of 5 or 10 mg/ℓ of HEX to sewage effluent inoculated with Salmonella typhosa was also

TABLE 6-3

Effects of 28 Days Exposure of Mysid Shrimp, *Mysidopsis bahia*, to HEX[a]

| Concentration (μg/ℓ) |  | Mortality (%) | Total Offspring | Offspring per Female |
|---|---|---|---|---|
| Nominal | Measured |  |  |  |
| Control | ND | 0 | 195 | 15.7 |
| 0.75 | 0.30 | 18.9 | 167 | 11.6 |
| 1.5 | 0.70 | 43.6[b] | 67 | 5.0[b] |
| 3.0 | 3.0 | 18.4[c] | 79 | 5.4[b] |
| 6.0 | 2.9 | 23.1 | 72 | 5.5[b] |
| 12.0 | 6.2 | 97.5[b] | 0 | 0[b] |

[a] Source: U.S. EPA, 1981

[b] Significantly different from the control ($p<0.05$)

[c] No explanation was given in original text as to this value in comparison with the next measured value of 2.9 μg/ℓ.

ND = Not detected

96 hours of contact, ranged from 1-10 mg/ℓ HEX. Addition of 5 or 10 mg/ℓ of HEX to sewage effluent inoculated with _Salmonella typhosa_ was also found to be more effective than similar concentrations of chlorine in reducing total bacterial count, coliforms and _S. typhosa_ (Cole, 1954). Yowell (1951) also reported in a patent application that HEX has antibacterial properties; standard phenol coefficients for _E. typhus_ (sic) and _Staphylococcus aureus_ were 25 and 33, at 21 and 23 ppm of HEX, respectively. These findings indicated that concentrations of HEX at or slightly above its aqueous solubility limit were toxic to several types of pathogens.

In contrast, tests with other microorganisms have shown some ability to withstand HEX exposure. Twenty-three strains of organisms (type unspecified), when added to aqueous medium containing HEX at 1000 mg/ℓ, were able to metabolize the compound to a varying degree. Analysis of the medium after 14 days indicated a HEX removal of 2-76%, depending on the organism used (Thuma et al., 1978).

Rieck (1977a) found no effects on natural populations of bacteria, actinomycetes and fungi after 24 days incubation of a sandy loam soil treated with 1 or 10 μg/g (dry weight) HEX. He concluded that no significant detrimental effects on microbial populations would result from treatment of soils with these levels of HEX.

The effects of HEX on three ecologically important microbial processes were recently reported by Velsicol (Butz and Atallah, 1980). Results on cellulose degradation by the fungus _Trichoderma longibrachiatum_ indicated that a suspension of HEX inhibited cellulose degradation at a concentration of 1 mg/ℓ and higher in a liquid medium. The calculated 7-day $EC_{50}$ was 1.1 mg/ℓ. Extrapolations for the 1- and 3-day $EC_{50}$ values were reported to be 0.2 mg/ℓ. The decrease in toxicity in the 7-day period was attributed to adaptation by _T. longibrachiatum_.

HEX inhibited anaerobic sulfate reduction by Desulfovibrio desulfuricans when present in suspension in a liquid medium. Following a 3-hour contact period, growth inhibition was observed at HEX concentrations of 10-100 mg/ℓ, and no growth was evident at 500 and 1000 mg/ℓ. Similarly, growth inhibition was observed at 1 and 10 mg/ℓ following a 24-hour contact period, and no growth was evident at 50-1000 mg/ℓ. HEX was considered slightly toxic to D. desulfuricans (Butz and Atallah, 1980).

A third study by the same investigators (Butz and Atallah, 1980) focused on the effects of HEX on urea ammonification by a mixed microbial culture in moist soil. The results indicated that HEX concentrations of 1-100 μg/g (dry weight) were not toxic to soil organisms responsible for urea ammonification. $EC_{50}$ increased from 104 μg/g at 1 day to 1374 μg/g at 14 days. The authors suggested that the low toxicity and its decrease over time in this experiment may have been due to adsorption of the toxicant onto soil particles, as well as to potential adaptation by the organism. Soil adsorption may also account for the lack of toxicity in the test by Reick (1977a).

## 6.3. EFFECTS ON TERRESTRIAL VEGETATION

In a patent application, HEX was reported to be nontoxic to plants in concentrations at which it was an effective fungicide (Yowell, 1951). Test solutions were prepared by adding HEX at various proportions to attaclay and a wetting agent, and the mixture was then mixed with water. The concentrations of HEX applied to plants as an aqueous spray were 0.1, 0.2, 0.5 and 1.0%. Slight injury (unspecified) to Coleus blumei was reported at 1.0% HEX, whereas lower concentrations were not harmful. Similarly, HEX was added to horticultural spray oil and an emulsifier at various proportions and then mixed with water. The concentations of HEX in the prepared spray were 0.25 and 0.5%. No injury to C. blumei was observed at these concentrations.

## 6.4. EFFECTS ON WILDLIFE

No data were available on the effects of HEX on amphibians, reptiles or birds, or on mammals other than those typically utilized in laboratory testing.

## 6.5. SUMMARY

The toxicity of HEX to several forms of aquatic life has been demonstrated. The freshwater cladoceran Daphnia magna gave 48-hour $LC_{50}$ values of 39 and 52 μg/ℓ in static tests (Buccafusco and LeBlanc, 1977; Vilkas, 1977). Freshwater fish species tended to be slightly more tolerant, with 96-hour $LC_{50}$ values ranging from 59-180 μg/ℓ (Henderson, 1956; Buccafusco and LeBlanc, 1977; Podowski and Khan, 1977). However, when fathead minnow fry (larvae) were tested in a flowing system, a value of 7 μg/ℓ was obtained (Spehar et al., 1977, 1979).

Saltwater crustaceans were of similar sensitivity as D. magna in static tests; 96-hour $LC_{50}$ values for two shrimp species were 32 and 42 μg/ℓ, while a polychaete was more resistant with a value of 371 μg/ℓ. However, a flowthrough test with mysid shrimp gave a 96-hour $LC_{50}$ of 7 μg/ℓ. Three saltwater fish species all had static $LC_{50}$ values within the range of 37-48 μg/ℓ (U.S. EPA, 1980a).

The chronic MATC for the fathead minnow, based on a 30-day early lifestage test, was between 3.7 and 7.3 μg/ℓ, as was the acute $LC_{50}$ (Spehar et al., 1977, 1979). Thus no cumulative toxic effect was observed, and there was also no accumulation of residues of HEX. Fish growth was unaffected in this test. On the other hand, a 28-day chronic test with mysid shrimp gave an MATC between 0.30 and 0.70 µg/ℓ, well below the acute value of 7 µg/ℓ for this species. Both survival and fecundity were reduced by toxicant exposure (U.S. EPA, 1981).

In the only tests conducted with aquatic plants, two of four saltwater unicellular algal species tested were of comparable sensitivity as crustaceans, with 7-day $EC_{50}$ values of 3.5 and 6.6 µg/ℓ, respectively. The other species were somewhat more tolerant (Walsh, 1981).

In general, flowing toxicant concentrations produced a greater response than static concentrations, and measured concentrations were found to be about one-half of nominal concentrations. Thus static tests, all based on nominal concentrations, probably underestimated HEX toxicity. Tests initiated with other than newborn animals could also have underestimated the toxic response of natural populations exposed to HEX.

In aqueous media, HEX is toxic to many microorganisms at nominal concentrations of 0.2-10 mg/ℓ, or levels substantially higher than those needed to kill most aquatic animals or plants (Cole, 1953, 1954; Yowell, 1951). Some microorganisms are able to withstand exposures as high as 1000 mg/ℓ (Thuma et al., 1979). HEX appears to be less toxic to microorganisms in soil than in aquatic media, probably because of adsorption on the soil matrix (Rieck, 1977a; Butz and Atallah, 1980).

Sufficient information is not available to determine the effects of HEX exposure on terrestrial vegetation or wildlife, although data from laboratory studies summarized in the following sections could be used to estimate effects on wild mammals.

# 7. TOXICOLOGY AND HEALTH EFFECTS

## 7.1. PHARMACOKINETICS

### 7.1.1. Absorption, Distribution, Metabolism and Excretion.

7.1.1.1. ORAL -- Mehendale (1977) studied the absorption, metabolism, excretion and tissue distribution of HEX in 225-250 g male Sprague-Dawley rats. A single dose of 6 mg/kg $^{14}$C-HEX in corn oil was given by oral gavage. The animals were maintained in metabolism cages for 7 days. Urine and fecal samples were collected daily. After 7 days, the rats were sacrificed and the amount of radiolabel in major organs, urine and powdered feces was determined. Ten percent of the radiolabel was recovered in the feces and 33% in the urine during the 7 days while only trace amounts were found in the liver, kidney and other major organs. Since >50% of the administered dose was not accounted for, the author speculated that the respiratory tract was the major route of excretion for orally administered HEX. Subsequent studies that are reviewed later in this chapter, in which exhaled air and lung tissues were analyzed for $^{14}$C activity, have shown that this is not the case. Another interpretation of these results is that HEX and/or its metabolites were volatilized and lost during sample preparation, i.e., powdering of the feces before analysis (Whitacre, 1978). Mehendale also studied the subcellular distribution of radiolabel in cellular fractions of rat liver and kidney following oral administration of $^{14}$C-HEX. In both organs, the majority of radiolabel was located in the cytosol. Specific metabolites and the metabolic form of the radiolabel in various fractions and samples were not identified in these studies.

In 1979, Dorough studied the absorption, tissue distribution and excretion of HEX in male and female Sprague-Dawley rats (200-250 g) and mice (strain not specified; 25-30 g). The animals were divided into two comparable groups and were given a single oral dose of 2.5 or 25 mg/kg of $^{14}$C-HEX (corn oil vehicle). The animals were placed in metabolism cages equipped with a trap to collect expired organocompounds and a trap to collect expired carbon dioxide. Less than 1% of the radiolabel was trapped in the expired gases over a 3-day period. The pattern of results for other routes of elimination was similar in both sexes of each species. Therefore, this study disproves Mehendale's (1977) speculation that the compound was mainly excreted by exhaled air. After 3 days, animals given 2.5 mg/kg excreted an average of 68% of the radiolabel in the feces and 15% in the urine while animals given 25 mg/kg excreted an average of 72% of the radiolabel in the feces and 14% in the urine. Total recovery of radiolabel was between 83 and 86%. Thus, 14-17% of the radiolabel was not accounted for in this study. In addition, Dorough fed 1, 5 or 25 ppm HEX to rats and mice for a maximum of 30 days. During this study, 54-70% of the radiolabel was excreted in the feces and 6-12% in the urine. The total cumulative recovery of radiolabel ranged between 63 and 79% with average values of 72% recovery. This means that an average of 28% of the radiolabel was left unaccounted. Metabolites were not identified in these studies.

In a study by Yu and Atallah (1981), male and female Sprague-Dawley rats (240-350 g) were given a single dose of 3 or 6 mg/kg $^{14}$C-HEX in 0.5 mℓ corn oil by gavage. Radioactivity appeared in the blood (taken from the tail) within 30 minutes, reached a maximum value at 4 hours, and then gradually decreased. Within 48 hours, 70% of the radiolabel was excreted in the feces and 17% in the urine while only a total of 2.8% was retained in the liver, kidneys, fat, muscle, brain and heart. Thus, ~90% of the radiolabel was recovered in this study. Metabolites were not identified by various chromatographic methods although the authors stated that no unchanged HEX (i.e., HEX that was not metabolized or bound to other molecules) was found in the excreta or tissues examined after killing the animals. When HEX was incubated *in vitro* with the contents of rat gut or with fecal homogenates, the estimated half-life of unchanged HEX was 10.1 hours and 1.6 hours, respectively. The addition of $HgCl_2$ to fecal homogenates and gut contents resulted in decreases in the degradation rates of HEX. On this basis, the authors concluded that HEX was poorly absorbed in the gut and that microbial action was responsible for the metabolism of HEX.

7.1.1.2. **DERMAL** -- There were no pharmacokinetic studies of HEX, involving the dermal route, found in our literature survey. While no quantitative studies of HEX absorbed through the skin were found, studies have been reported in which discoloration of the skin was observed following the dermal application of HEX (Treon et al., 1955; IRDC, 1972). Although this does not prove absorption, toxic response leading to death was observed in several cases. This fact would suggest that HEX is possibly absorbed trans-dermally into the systemic circulation. These studies are discussed in greater detail later in this chapter.

7.1.1.3. **INTRAVENOUS** -- Mehendale (1977) studied biliary excretion following injection of 1 μCi HEX (5μmole vehicle not identified) into the femoral vein or artery in Sprague-Dawley rats whose common bile duct had been cannulated. There was biexponential decay of radiolabel from the blood with estimated half-lives of ~5 and 60 minutes. Approximately 9% of the radiolabel was excreted in the bile in 1 hour.

Yu and Atallah (1981) administered 0.73 mg/kg $^{14}C$-HEX (10.6 mCi/mmole in 0.3 mℓ of 20% Emulphor® EL 0620 vehicle in saline solution) intravenously into the lateral caudal vein of Sprague-Dawley rats. Within 48 hours, 21% of the radiolabel was excreted in the feces and 18% in the urine while a total of ~28% of the radiolabel remained in the liver, kidneys, fat, muscle, brain and heart. Metabolites were not identified in this study and only 67% of the dose was recovered.

7.1.1.4. **INHALATION** -- In 1980, Dorough studied the absorption and fate of inhaled HEX in female Sprague-Dawley rats (175-250 g). Animals were exposed to vapors of $^{14}$C-HEX over a 1-hour period to achieve desired dosing of ~24 μg/kg body weight (both measured and from theoretical calculations). Considerable difficulty was experienced in maintaining the desired concentration of HEX throughout the exposure period. Approximately 69% of the radiolabel was recovered, with 13% in the body tissues, 23% in the feces, and 33% in the urine. Less than 1% of the inhaled radiolabel was recovered in the expired air following exposure.

These results were confirmed in a study by Lawrence and Dorough (1982) in which female Sprague-Dawley rats (175-225 g) were exposed in a specially-designed facemask system for 1 hour to concentrations ~24 μg/kg $^{14}$C-HEX. Retained doses received by rats during inhalation exposures ranged from 1-40 μg/kg bw, but the retention of inhaled $^{14}$C-HEX was not influenced by the quantity received within this range of doses (Lawrence and Dorough, 1982). Following exposure, <1% of the recovered radiolabel was expired as organo-compounds and no detectable $^{14}$C-carbon dioxide was expired. The trachea and lungs contained the highest levels of radiolabel with 107 and 74.5 ng equivalent/g tissue, respectively. Radioacarbon remaining in the body after 72 hour represented 12.9 and 31.0% of the inhalation and i.v. treatments.

In their experiment studying the effects of HEX exposure on the Clara cells of monkeys and rats, Rand et al. (1982b) hypothesized that HEX vapor inhalation interferes with metabolism by the peroxidation of membrane-bound unsaturated lipids. These researchers suggest that there would be a decrease in the production of pulmonary cytochrome P-450, resulting in a modification of the microsomal enzyme system of the smooth endoplasmic reticulum to metabolize foreign compounds. This resultant biochemical action then changes the morphology of the secretory glands.

7.1.1.5. **COMPARATIVE STUDIES** -- In the inhalation studies of El Dareer et al. (1983), Dorough (1980) and Lawrence and Dorough (1982), and groups of rats were given HEX by oral gavage and by intravenous (i.v.) injection in order to compare the results for the three routes of administration. Tables 7-1, 7-2 and 7-3 summarize the results of these three studies. The tissue distribution was different for the three routes of administration. The results of the oral studies compare quite favorably with the studies of Dorough (1979) and Yu and Atallah (1981).

El Dareer et al. (1983) completed a HEX disposition comparison study using male Fischer 344 rats for the National Toxicology Program (NTP). HEX (95-99% pure) was administered orally (4.1 and 61 mg/kg), intravenously (0.59 mg/kg) and by inhalation (1.0 and 1.4 mg/kg). The disposition of radioactivity from $^{14}$C-HEX in rats dosed by various routes is summarized in Table 7-1. In this experiment after oral doses, most of the radioactivity appeared in the urine and feces within 72 hours. In comparing the oral with the i.v. route, the percentages found in the urine and feces were smaller with a comparitively large proportion of the radioactivity remaining in the tissues, mostly in the liver and carcass. The rats exposed to the vapor had a higher percentage remaining in the tissues as compared with oral dosing, but lower in comparison with the i.v. route. Metabolities were not identified in the study.

## TABLE 7-1

Disposition of Radioactivity Expressed as Percentage of Administered Dose from $^{14}$C-HEX in Rats Dosed by Various Routes[a]

| | Oral Dose | | Intravenous Dose[b] | Inhalation Dose | |
|---|---|---|---|---|---|
| | Low Dose[b] (4.1 mg/kg) | High Dose[b] (61 mg/kg) | 0.59 mg/kg | Group A[c] (1.0 mg/kg) | Group B[b] (1.4 mg/kg) |
| Feces | 79.4 ± 2.9% | 65.3 ± 6.9% | 34.0 ± 1.0%[d] | 28.7 ± 4.3% | 47.5 ± 6.4% |
| Urine | 35.5 ± 2.5% | 28.7 ± 4.2% | 15.8 ± 1.4% | 41.0 ± 4.8% | 40.0 ± 6.6% |
| Tissues | 2.4 ± 0.6% | 2.4 ± 0.1% | 39.0 ± 1.0% | 28.9 ± 1.6% | 11.5 ± 0.8% |
| $CO_2$ | 0.8 ± 0.0% | 0.6 ± 0.0% | 0.1 ± 0.0% | 1.4 ± 0.3% | 1.0 ± 0.5% |
| Other volatile | 0.2 ± 0.0% | 0.3 ± 0.0% | 0.1 ± 0.0% | -- | - |
| TOTAL RECOVERY | 118.0 ± 3.0%[e] | 97.0 ± 7.0% | 89.0 ± 2.0% | (100%) | (100%) |

[a] Source: Adapted from El Dareer et al., 1983 (The values represent the mean % of dose ± standard deviation for three rats.

[b] At 72 hours after dosing or exposure

[c] At 6 hours after exposure

[d] Plus intestinal contents

[e] For an unexplained reason, the total recovery for this dose was higher than theoretical. If the percent recoveries for this dose are "normalized" to 100%, differences in disposition for the two doses are minimal, an indication that no saturable process is operative in this dose range.

## TABLE 7-2

### Fate of Radiocarbon Following Oral, Inhalation and Intravenous Exposure to $^{14}$C-HEX in Rats Expressed as Percentage of Administered Dose[a]

| | Cumulative Percent of Dose | | |
|---|---|---|---|
| | Oral[b] | Intravenous[c] | Inhalation[d] |
| | **24-Hour** | | |
| Urine | 22.2 ± 1.8 | 18.3 ± 5.2 | 29.7 ± 4.5 |
| Feces | 62.2 ± 8.0 | 21.1 ± 7.1 | 17.0 ± 7.5 |
| | **48-Hour** | | |
| Urine | 24.0 ± 1.9 | 20.7 ± 5.6 | 32.5 ± 5.1 |
| Feces | 67.7 ± 5.1 | 30.4 ± 1.7 | 21.0 ± 7.5 |
| | **72-Hour** | | |
| Urine | 24.4 ± 1.9 | 22.1 ± 5.7 | 33.1 ± 4.5 |
| Feces | 68.2 ± 5.1 | 47.4 ± 1.9 | 23.1 ± 5.7 |
| Body | 0.2 ± 0.2 | 15.7 ± 7.8 | 12.9 ± 4.7 |
| Total Recovery | 92.8 ± 4.7 | 85.2 ± 4.8 | 69.1 ± 9.6 |

[a] Source: Adapted from Dorough, 1980, and Lawrence and Dorough, 1982

[b] Doses administered in 0.5 mℓ corn oil at 7 μg/kg body weight

[c] Doses administered in 0.2 mℓ 10:4:1 saline:propylene glycol:ethanol by injection into the femoral vein at 5 μg/kg body weight

[d] Doses administered as vapors over a 1-hour exposure period to achieve doses of ~24 μg/kg body weight.

## TABLE 7-3

Distribution of HEX Equivalents[a] in Tissues and Excreta of Rats 72 Hours After Oral, Inhalation and Intravenous Exposure to $^{14}$C-HEX[b,c]

| Sample | Oral Dose (6 mg/kg)[d] | Inhaled Dose (~24 μg/kg) | Intravenous Dose (10 μg/kg) |
|---|---|---|---|
| | | ng/g of Tissue | |
| Trachea | 292 ± 170 | 107.0 ± 65.0 | 3.3 ± 1.7 |
| Lungs | 420 ± 250 | 71.5 ± 55.2 | 14.9 ± 1.1 |
| Liver | 539 ± 72 | 3.6 ± 1.9 | 9.6 ± 1.1 |
| Kidneys | 3272 ± 84 | 29.5 ± 20.2 | 22.3 ± 0.6 |
| Fat | 311 ± 12 | 2.8 ± 0.4 | 2.3 ± 0.2 |
| Remaining carcass | 63 ± 40 | 1.3 ± 0.6 | 0.5 ± 0.1 |
| | | Percent of Dose | |
| Whole Body | 2.8 ± 1.1 | 12.9 ± 4.7 | 31.0 ± 7.8 |
| Urine | 15.3 ± 3.3 | 33.1 ± 4.5 | 22.1 ± 5.7 |
| Feces | 63.6 ± 8.5 | 23.1 ± 5.7 | 31.4 ± 1.9 |
| Total Recovery | 81.7 ± 6.7 | 69.1 ± 9.6 | 84.6 ± 4.6 |

[a] One HEX equivalent is defined as the amount of radiolabel equivalent to one nanogram of HEX based on the specific activity of the dosing solution.

[b] Source: Adapted from Dorough, 1980 and Lawrence and Dorough, 1982

[c] All values are the Mean ± S.D. of three replicates.

[d] Note that the oral dose was 250 and 600 times that of the inhaled and i.v. doses, respectively. That was necessary since residues were not detected in individual tissues of animals treated orally at doses of 5-25 μg/kg.

This study (El Dareer et al., 1983) confirms Dorough's studies in that the major routes of elimination are fecal and urinary. Little radioactivity appeared as $^{14}CO_2$ or as other volatile compounds. Since little radioactivity was detected in the exhaled air, the respiratory tract is not a substantial route of elimination of HEX. This substantiates the findings of Lawrence and Dorough (1981) and negates the Mehendale (1977) conclusion. The radioactivity found in the urine, feces and body after 72 hours were similar to Lawrence and Dorough (1981) with the exception of a higher percentage being found in the feces than in the urine.

Several observations have been made during the development and peer review of this document. During inhalation and the passage of HEX through the lung tissue to reach the blood, metabolism to water-soluble compounds may occur and HEX would be eliminated through the kidneys. In contrast, an i.v. dose may be bound unchanged to blood components and remain attached until reaching the liver, whereupon it may be displaced and become associated with the liver tissue. However, Lawrence and Dorough (1982) still conclude that regardless of the route of HEX administration, damage to the lungs occurs and in all cases appears to be the primary cause of death in the laboratory animals.

**7.1.1.6. CONCLUSIONS REGARDING THE FATE OF HEX IN BIOLOGICAL SYSTEMS** -- From the data presented in the pharmacokinetic studies, the following points can be made regarding the fate of HEX in biological systems:

- HEX or its metabolites interact with biological tissues as indicated by the following:
    - high concentrations of HEX are found in the lung and trachea following inhalation exposure, skin darkens in appearance when exposed, and HEX interacts, at a fairly rapid rate, with gut and fecal homogenates

- HEX is not readily absorbed through the gastrointestinal tract as indicated by the following:
  - there is a high retention of HEX in the fecal contents of animals dosed orally and there is relatively little biliary excretion to account for this dose
- HEX equivalents are not volatilized and lost in expired air during the first 72 hours following dosing as indicated by the following:
  - no radiolabelled carbon dioxide and only small amounts of $^{14}C$-HEX were found in animals post exposure after dosing by the pulmonary, i.v. or oral routes

Since the recovery of radiolabel following HEX administration varies from 43% to >90% in the pharmacokinetic studies reported, a need for a more thorough study of the pharmacokinetics of HEX by various exposure routes is evident. A major portion of the radiolabel may be "fixed" to tissues at the site of administration and missed in routine recovery procedures for pharmacokinetics studies. No one has measured the amount of radiolabel retained by the blood vessel walls or the gastrointestinal epithelial tissues. One might expect binding to these tissues (as sites of uptake) after i.v. or oral dose administration.

7.1.2. **Summary.** Pharmacokinetic studies designed to determine the absorption, distribution, metabolism and elimination of HEX in rats and mice have involved the oral, i.v. and inhalation routes of administration of $^{14}C$-HEX. The fecal excretion of radiolabel following oral dosing is 2- to 3-fold higher than for i.v. or inhalation administration which indicates that HEX is not readily absorbed from the gastrointestinal tract. Following inhalation, considerable radiolabel remained in the lung and trachea indicating that HEX reacts with biological membranes and molecules _in vivo_. HEX has also been shown to react with the contents of the gastrointestinal tract _in vitro_. Since up to 57% of the radiolabel has not been accounted for even in studies in which considerable effort has been made to recover all of the radiolabel, HEX might possibly react with biological membranes and molecules at all sites of administration or membrane transport. A number of studies have been conducted to elucidate the whereabouts of HEX in body tissues after exposure by different routes. However, since the $^{14}C$-labelled compound used in these studies did not allow for the identification of any of the metabolites, little, as yet, is known about the fate of HEX or its metabolites.

## 7.2. MAMMALIAN TOXICOLOGY

7.2.1. **Acute Toxicity.** The acute toxicity of HEX is summarized in Table 7-4. A complete toxicity table is also presented in Appendix 1.

7.2.1.1. **ACUTE ORAL TOXICITY** -- Treon et al. (1955) conducted a series of oral toxicity studies using female rabbits (strain unspecified) and Carworth rats of both sexes. HEX was administered as a 5% solution in peanut oil by oral gavage. The oral $LD_{50}$ for female rabbits was determined to be ~640 mg/kg. The oral $LD_{50}$ for male and female rats was ~510 mg/kg and 690 mg/kg, respectively. In 1968, IRDC determined the oral $LD_{50}$ for albino rats to be 926 mg/kg for HEX given in corn oil by oral gavage. In more recent studies, Dorough (1979) reported the oral $LD_{50}$ for male and female Sprague-Dawley rats to be ~651 mg/kg and for male and female mice (strain unspecified) to be greater than 600 mg/kg. Thus, HEX is moderately toxic when given orally. Based on FIFRA guidelines (40 CFR 162.10) HEX, when administered orally to young adult experimental animals, would be classified in Toxicity Category III. In addition, Southern Research Institute (SRI, 1980a) reported the oral $LD_{50}$ for male and female weanling $B_6C_3F_1$ mice to be 680 mg/kg. Also, SRI (1980a) reported the oral

TABLE 7-4

Acute Toxicity of HEX

| Study/Reference | Species/Age | Results | Toxicity* Category |
|---|---|---|---|
| Oral $LD_{50}$/<br>Treon et al., 1955 | Rat, young adult | $LD_{50}$: Males - 510 mg/kg<br>Females - 690 mg/kg | III<br>III |
| Oral $LD_{50}$/<br>Treon et al., 1955 | Rabbit, adult | $LD_{50}$: Females - 640 mg/kg | III |
| Oral $LD_{50}$/<br>IRDC, 1968 | Rat, young adult | $LD_{50}$: Males and Females - 926 mg/kg | III |
| Oral $LD_{50}$/<br>Dorough, 1979 | Rat, young adult | $LD_{50}$: Males and Females - 651 mg/kg | III |
| Oral $LD_{50}$/<br>Dorough, 1979 | Mouse, young adult | $LD_{50}$: Males and Females - 600 mg/kg | III |
| Oral $LD_{50}$/<br>SRI 1980a | Rat, weanling | $LD_{50}$: Males - 425 mg/kg<br>Females - 315 mg/kg | II<br>II |
| Oral $LD_{50}$/<br>SRI, 1980a | Mouse, weanling | $LD_{50}$: Males and Females - 680 mg/kg | III |
| Dermal $LD_{50}$/<br>Treon et al., 1955 | Rabbit, adult | $LD_{50}$: Females - 780 mg/kg | II |
| Dermal $LD_{50}$/<br>IRDC, 1972 | Rabbit, adult | $LD_{50}$: Males - 200 mg/kg<br>Females - 340 mg/kg | II<br>II |

TABLE 7-4 (cont.)

| Study/Reference | Species/Age | Results | Toxicity* Category |
|---|---|---|---|
| Inhalation $LC_{50}$/ Treon et al., 1955 | Rat, young adult | 3.5-hour $LC_{50}$: Males and Females - 3.1 ppm | I |
| Inhalation $LC_{50}$/ Rand et al., 1982 | Rat, young adult | 4-hour $LC_{50}$: Males - 1.6 ppm<br>Females - 3.5 ppm | I<br>I |
| Inhalation $LC_{50}$/ Treon et al., 1955 | Rabbit, adult | 3.5-hour $LC_{50}$: Females - 5.2 ppm | II |
| Inhalation $LC_{50}$/ Treon et al., 1955 | Guinea pig, young adult | 3.5-hour $LC_{50}$: Males and Females - 7.1 ppm | II |
| Inhalation $LC_{50}$/ Treon et al., 1955 | Mouse, adult | 3.5-hour $LC_{50}$: Males and Females - 2.1 ppm | I |
| Primary Eye Irritation/ IRDC, 1972 | Rabbit, adult | Severe eye irritant (0.1 mℓ for 5 minutes or 24 hours) all dead by day 9 of study | I |
| Primary Dermal Irritation/ Treon et al., 1955 | Rabbit, adult | Moderate skin irritant (250 mg/kg) One application | II |
| Primary Dermal Irritation/ IRDC, 1972 | Rabbit, adult | Severe skin irritant (200 mg/kg). All males died in study | II |
| Primary Dermal Irritation/ Treon et al., 1955 | Monkey, adult | Mild skin discoloration (0.05 mℓ of 10% HEX solution) | None |

*According to the FIFRA guidelines, 40 CFR 162.10

$LD_{50}$ for weanling Fischer 344 rats to be 425 mg/kg for males and 315 mg/kg for females.

7.2.1.2. **ACUTE DERMAL TOXICITY** -- Treon et al. (1955) reported the dermal $LD_{50}$ in female rabbits (strain unspecified) to be 780 mg/kg while IRDC (1972) reported the dermal $LD_{50}$ in albino rabbits (strain unspecified) to be <200 mg/kg in males and to be 340 mg/kg in females. These data would place HEX, when applied dermally, in Toxicity Category II.

7.2.1.3. **ACUTE INHALATION TOXICITY** -- Treon et al. (1955) reported a 3.5-hour $LC_{50}$ of 3.1 ppm for Carworth rats of both sexes. Rand et al. 1982a reported a 4-hour $LC_{50}$ of 1.6 ppm for male Sprague-Dawley rats and 3.5 ppm for female rats. Treon et al. (1955) determined the 3.5-hour $LC_{50}$ to be 5.2 ppm in female rabbits, 2.1 in male and female mice, and 7.1 in male and female guinea pigs. These concentrations are in the range of 0.02-0.08 mg/ℓ for HEX vapor for rats and mice which would place HEX, when inhaled, in Toxicity Category I.

7.2.1.4. **EYE IRRITATION** -- IRDC (1972) tested HEX for eye iritation by instilling 0.1 mℓ HEX into the eyes of New Zealand white rabbits for 5 minutes or 24 hours before washing. All rabbits died on or before the 9th day of the observation period. HEX is a strong eye irritant and would be in Toxicity Category I based on ocular exposure.

7.2.1.5. **DERMAL IRRITATION** -- Treon et al. (1955) reported HEX to be a primary skin irritant in rabbits (strain unspecified) at a dose level of 250 mg/kg. In 1972, IRDC reported HEX in New Zealand white rabbits to be a dermal irritant based upon edema observed following application of 0.5 mℓ HEX. In this study, intense discoloration of the skin was noted. These data would place HEX in Toxicity Category II for dermal irritation. In the

Treon study (1955), monkeys (strain unspecified) were also tested and discoloration of the skin was noted even at low doses (0.05 mℓ of 10% HEX).

7.2.1.6. **SUMMARY** -- The acute oral toxicity of HEX has been studied in rats, rabbits and mice. The oral $LD_{50}$ for adult animals is >500 mg/kg which places HEX in Toxicity Category III. The acute dermal toxicity of HEX has been studied in rabbits and, because <50% of the animals died at the tested dose, the dermal $LD_{50}$ is >200 mg/kg which places HEX in Toxicity Category II. The acute inhalation toxicity of HEX has been studied in rats, rabbits, guinea pigs and mice. In rats and mice, the 3.5-4.0 hour $LC_{50}$ for HEX is <0.2 mg/ℓ which places HEX in Toxicity Category I. In comparison, the pathological effects are observed in the lung no matter which route of administration of HEX is used. In addition, HEX is a severe eye, skin and pulmonary irritant.

### 7.2.2. Subchronic Toxicity.

7.2.2.1. SUBCHRONIC ORAL TOXICITY --

7.2.2.1.1. Range-Finding Studies -- Using small range-finding tests Litton Bionetics (1978b) determined the oral $LD_5$ of HEX in CD-1 mice to be 76 mg/kg. However, when this expected $LD_5$ was administered to mice for 5 consecutive days, all mice (24) died within the 5-day period. In a range-finding study using groups of 5 male and 5 female Fischer 344 rats, SRI (1980a) reported no mortality at doses of 25, 50 or 100 mg/kg when given 12 doses in 16 days. At 200 mg/kg and using the same dosing schedule, 5 of 5 males and 4 of 5 females died, and at 400 mg/kg, 5 of 5 males and 4 of 5 females died during the study. In the same study, $B6C3F_1$ mice died when given doses of 400 or 800 mg/kg but not at doses of 50, 100 or 200 mg/kg. Both rats and mice exhibited pathologic changes of the stomach wall in all but the lowest dose level.

**7.2.2.1.2. Studies 90 Days or Longer in Duration** -- The subchronic toxicity of HEX is summarized in Table 7-5. Subchronic toxicity studies in B6C3F$_1$ mice and Fischer 344 rats have been conducted by SRI (1981a,b) under contract with the National Toxicology Program (NTP). In the mouse study (1981a), dose levels of 19, 38, 75, 150 and 300 mg/kg HEX (94.3-97.4%) in corn oil were administered by gavage to 10 mice of each sex, 5 days/week for 13 weeks (91 days). At the highest dose level (300 mg/kg), all male mice died by day 8 and three females died by day 14. In female mice, the liver was enlarged. Toxic nephrosis in females at doses of 75 mg/kg and higher was characterized by lesions in the terminal portions of the convoluted tubules, with basophilia in the inner cortical zone and cytoplasmic vacuolization. However, male mice at this level and higher did not show these effects. Dose levels of 38 mg/kg HEX and above caused lesions in the forestomach, including ulceration in both males and females. The no observed adverse effect level (NOAEL) in mice for HEX was 19 mg/kg and the lowest observed effect level (LOEL) was 38 mg/kg.

In the rat study (SRI, 1981b), dose levels of 10, 19, 38, 75 and 150 mg/kg HEX in corn oil were administered by gavage to groups of 10 male and female F344 rats. At the 38 mg/kg dose and higher levels, mortality and toxic nephrosis were noted in both males and females. The male rats treated at the 19 mg/kg dose level showed no highly abnormal effects while female rats exhibited lesions of the forestomach. Such lesions were observed in males at 38 mg/kg or higher levels. There was a dose-related depression of body weight gain relative to the controls. The NOAEL in rats for HEX was 10 mg/kg and the LOEL was 19 mg/kg.

A summary of the results of these two experiments appears in Table 7-6. Based on these studies, a maximum tolerated dose (MTD) of 38 mg/kg for mice

## TABLE 7-5

### Subchronic Toxicity of HEX

| Study/Reference | Species | Dose | Results | Effects at LOEL or Lowest Dose |
|---|---|---|---|---|
| 90-Day Feeding Study/ SRI, 1981b | Rat | 10, 19, 38, 75, 150 or 300 mg/kg (by gavage) | NOAEL - 10 mg/kg<br>LOEL - 19 mg/kg | Lesions of forestomach in female rats at 19 mg/kg |
| 90-Day Feeding Study/ SRI, 1981a | Mouse | 19, 38, 75, 150 or 300 mg/kg (by gavage) | NOAEL - 19 mg/kg<br>LOEL - 38 mg/kg | Lesions of forestomach in both sexes at 38 mg/kg |
| 14-Week Inhalation Toxicity Study/ Rand et al., 1982 | Rat | 0.01, 0.05 and 0.2 ppm (5 days/week) | NOEL - 0.2 ppm<br>LOEL - NE | No statistically significant effects |
| 14-Week Inhalation Toxicity Study/ Alexander et al., 1980 | Monkey | 0.01, 0.05 and 0.2 ppm (5 days/week) | NOEL - 0.2 ppm<br>LOEL - NE | No effects noted |

NE - Not established

## TABLE 7-6

### Toxicological Parameters for Mice and Rats Administered Technical Grade HEX in Corn Oil for 91 Days[a]

| Species/ Strain | Dose (mg/kg) | Mortality | Relative Weight Gain[b] | Pathology | | |
|---|---|---|---|---|---|---|
| | | | | Forestomach | | Kidney |
| | | | | Inflammation | Hyperplasia | Toxic Nephrosis |
| Male mice/ B6C3F$_1$ | 0 | 1/10 | -- | 0/10 | 0/10 | 0/10 |
| | 19 | 0/10 | +36% | 0/10 | 0/10 | 0/10 |
| | 38 | 0/10 | + 9% | 2/10 | 2/10 | 0/10 |
| | 75 | 0/10 | - 9% | 7/10 | 8/10 | 0/10 |
| | 150 | 0/10 | -45% | 7/10 | 9/10 | 0/10 |
| | 300 | 10/10 | -- | 7/10 | 8/10 | 0/10 |
| Female mice/ B6C3F$_1$ | 0 | 0/10 | -- | 0/10 | 0/10 | 0/10 |
| | 19 | 0/10 | +13% | 0/10 | 0/10 | 0/10 |
| | 38 | 0/10 | -13% | 2/9 | 2/9 | 0/9 |
| | 75 | 0/10 | -13% | 6/10 | 9/10 | 10/10 |
| | 150 | 0/10 | -25% | 10/10 | 10/10 | 10/10 |
| | 300 | 3/10 | -38% | 7/9 | 9/9 | 7/10 |
| Male rats/ Fischer 344 | 0 | 3/10 | -- | 0/10 | 0/10 | 0/10 |
| | 10 | 1/10 | - 4% | 0/10 | 0/10 | 0/10 |
| | 19 | 1/10 | - 8% | 0/10 | 0/10 | 0/10 |
| | 38 | 1/10 | -20% | 4/10 | 5/10 | 10/10 |
| | 75 | 3/10 | -49% | 9/10 | 9/10 | 9/10 |
| | 150 | 7/10 | -57% | 8/9 | 8/9 | 8/10 |

TABLE 7-6 (cont.)

| Species/ Strain | Dose (mg/kg) | Mortality | Relative Weight Gain[b] | Pathology | | |
|---|---|---|---|---|---|---|
| | | | | Forestomach | | Kidney |
| | | | | Inflammation | Hyperplasia | Toxic Nephrosis |
| Female rats/ Fischer 344 | 0 | 1/10 | 0% | 0/10 | 0/10 | 0/10 |
| | 10 | 2/10 | + 4% | 0/10 | 0/10 | 0/10 |
| | 19 | 1/10 | - 5% | 2/10 | 2/10 | 0/10 |
| | 38 | 1/10 | - 2% | 2/10 | 5/10 | 10/10 |
| | 75 | 3/10 | -30% | 9/10 | 9/10 | 10/10 |
| | 150 | 5/10 | -33% | 9/10 | 9/10 | 10/10 |

[a] Source: Southern Research Institute, 1981a,b

[b] Relative weight gain is calculated as:

$$\frac{\text{Dose Group Value - Control Group Value}}{\text{Control Group Value}} \times 100.$$

and 19 mg/kg for rats was recommended by SRI to NTP for a chronic toxicity study.

#### 7.2.2.2. SUBCHRONIC DERMAL TOXICITY --

7.2.2.2.1. **Range-Finding Study** -- In a Russian study, Naishtein and Lisovskaya (1965) studied the effects of HEX applied to the shaved area of the skin of rabbits (strain unspecified) daily for 10 days. According to the authors, no effects were noted in control and test animals given daily doses of 0.5-0.6 mℓ of a 20 mg/ℓ solution of HEX.

#### 7.2.2.3. SUBCHRONIC INHALATION TOXICITY --

7.2.2.3.1. **Range-Finding Studies** -- Rand et al. (1982a) conducted a range-finding study in which groups of 10 male and 10 female Sprague-Dawley rats were exposed to atmospheres 0.022, 0.11 or 0.5 ppm HEX, 6 hours/day, 5 days/week for a total of 10 exposures. Nine male rats and one female rat exposed to 0.5 ppm HEX were moribund after 5-7 exposures. These rats had dark red eyes, labored breathing, and paleness of extremities. No mortalities were noted in the other exposure groups; however, the males in the 0.11 and 0.5 ppm groups lost weight during the study and alterations in liver weight and pathology were noted. The NOAEL for HEX exposure was 0.022 ppm and the LOEL was 0.11 ppm.

7.2.2.3.2. **Studies 90 Days or Longer in Duration** -- Fourteen-week inhalation studies in rats and monkeys have been performed (Rand et al., 1982a,b; Alexander et al., 1980). Groups of 40 male and 40 female Sprague-Dawley rats, weighing 160-224 g or groups of 12 Cynomolgus monkeys, weighing 1.5-2.5 kg, were exposed to HEX, 6 hours/day, 5 days/week, for as long as 14 weeks. Levels of exposure were 0, 0.01, 0.05 and 0.20 ppm HEX. In monkeys, there were no mortalities, adverse clinical signs, weight gain changes, pulmonary function changes, eye lesions, hematologic changes, clinical chemistry abnormalities or histopathologic abnormalities at any dose level tested. Thus, the no observed effect level (NOEL) for monkeys was 0.2 ppm HEX and the LOEL was not determined.

Male rats had a transient appearance of dark-red eyes at 0.05 and 0.2 ppm HEX. At 12 weeks, there were marginal but not statistically significant increases in hemoglobin concentration and erythrocyte count in 0.01 ppm males, 0.05 ppm females, and 0.20 ppm males and females. There were small but not statistically significant changes in mean liver weight of all treatment groups and similar changes in the kidneys of all treated males. There were no treatment-related abnormalities in gross pathology or histopathology. On this basis, the NOEL in rats was 0.2 ppm HEX; the LOEL was not established.

In the other study by Rand and coworkers (Rand et al., 1982b), no ultrastructural changes were observed that could be attributed to the inhalation of HEX vapor in exposed monkeys. Exposure was identical to that of the previous study (Rand et al., 1982a). This study took an in-depth look at the Clara cells and the results show a statistically significant ($p<0.01$) increase in the mean number of electron-lucent inclusions in the apex and base of the Clara cells in the exposed animals as compared with the controls. According to some researchers (Evans et al., 1978), Clara cells respond to injury by regression to a more primitive cell type. Rand et al. (1982b) noted that some of the ultrastructural changes in the exposed animals resemble those of the Evans study. It is not known what effect these changes might cause. The Clara cell contributes important materials to the extracellular lining of the peripheral airways, and if this effect from HEX vapors causes the content of the contributed material to be changed, then the extracellular lining may be altered and breathing may be subsequently impaired (Rand et al., 1982b). This observation coincides with those of other researchers (Dorough, 1979, 1980; Lawrence and Dorough, 1981, 1982). Furthermore, in the inhalation experiments with HEX, researchers have noted occasional statistically significant increases in hemoglobin and red blood cells of rats, which may be manifestations of the impairment of respiratory functions.

7.2.2.4. **SUMMARY** -- The subchronic toxicity of HEX has been studied in rats and mice following oral gavage and in rats and monkeys following inhalation exposure. In oral studies, rats and mice exhibited decreased body weight gain, lesions of the forestomach, and toxic nephrosis. Female mice also exhibited enlarged livers. The oral LOEL was 38 mg/kg for mice and 19 mg/kg for rats. In the inhalation studies, no abnormalities were observed in monkeys at doses as high as 0.2 ppm HEX for 6 hours over 14 weeks. No statistically significant changes were noted in blood parameters, and in kidney and liver weight in rats at all doses tested (range 0.01-0.2 ppm HEX). Thus, the NOEL in both rats and monkeys was 0.2 ppm; no LOEL was established.

### 7.2.3. Chronic Toxicity.

7.2.3.1. **CHRONIC ORAL TOXICITY** -- A chronic oral toxicity study of HEX being conducted by SRI for the National Toxicology Program was terminated in April 1982 because inhalation was determined to be the more relevant route of exposure. No other chronic oral toxicity data were available for this report.

7.2.3.2. **CHRONIC DERMAL TOXICITY** -- There were no chronic dermal toxicity studies found in the available literature.

7.2.3.3. **CHRONIC INHALATION TOXICITY** -- Treon et al. (1955) exposed guinea pigs, rabbits, rats and mice to a concentration of 0.33 ppm HEX for 7 hours/day, 5 days/week for 25-30 exposures. Guinea pigs survived 30 exposures; however, rats and mice did not survive 5 exposures and 4 of 6 rabbits did not survive 25 exposures. Using a lower concentration (0.15 ppm HEX), guinea pigs, rabbits and rats survived 150 seven-hour exposure periods (7 months). This level was too high for a chronic study in mice since 4/5 animals did not survive. The rats, guinea pigs and rabbits tolerated 0.15 ppm and did not exhibit any treatment-related effects. Thus, the NOEL for rats, guinea pigs and rabbits and the LOEL for mice was 0.15 ppm HEX. The NOEL for mice was not established while the LOEL for rats, guinea pigs and rabbits was 0.33 ppm HEX.

A 30-week chronic inhalation study of technical grade HEX in rats, 96% pure with hexachlorobuta-1,3-diene and octachlorocyclopentene as impurities, was conducted by Shell Toxicology Laboratory (D. Clark et al., 1982). Four groups of 8 male and 8 female Wistar albino rats were exposed to HEX at nominal concentrations of 0, 0.05, 0.1 and 0.5 ppm for 6 hours/day, 5 days/week, for 30 weeks and were observed for a 14-week recovery period without HEX exposure. At the highest dose level 4 males and 2 females died. In males, there was a depressed body weight gain in the 0.5 ppm group relative to controls beginning at 7 weeks of exposure and persisting throughout the remainder of the study. Females in the high and medium dose groups had lower body weights at the end of the recovery period as compared with the controls. At 0.5 ppm, there were pulmonary degenerative changes noted in both sexes although the males were affected more severely. At the highest dose, there were mild degenerative changes in the liver and kidneys at 30 weeks in a few rats and kidney weights were significantly increased in the females. After 30 weeks of study, there was no biologically significant toxicity noted in animals exposed to concentrations of 0.05 or 0.1 ppm HEX

(D. Clark, et al., 1982). Thus, the NOEL in rats exposed to vapors of HEX was 0.05 ppm; the LOEL was 0.1 ppm based on body weight, organ weight, and histopathology data.

A chronic inhalation study of HEX has been scheduled by the National Toxicology Program (Abdo, 1983).

7.2.3.4. **SUMMARY** -- The chronic effects of HEX have been studied primarily by inhalation exposure. No oral studies and one under-reported dermal study were located for this review. The inhalation toxicity of HEX has been evaluated in rats, mice, rabbits and guinea pigs. Four of five mice did not survive exposure to 0.15 ppm HEX, while the other species did not show effects following 150 seven-hour exposures to 0.15 ppm. In a more recent study, chronic degenerative changes in the lung, liver and kidneys were noted in rats exposed to 0.5 ppm HEX and the NOEL for rats was 0.05 ppm HEX. A 2-year inhalation bioassay has been scheduled by the National Toxicology Program to begin in 1984 (Abdo, 1983).

## 7.3. MUTAGENICITY

7.3.1. **Mutagenicity.** Goggelman et al. (1978) found that HEX was not mutagenic before or after liver microsomal activation at $2.7x10^{-3}$ M in an *E. coli* $K_{12}$ back mutation system. In this test there was 70% survival of bacteria at 72 hours. HEX was not tested at higher concentrations because it was cytotoxic to *E. coli*. A previous report from the same laboratory (Greim et al., 1977) indicated that HEX was also not mutagenic in *S. typhimurium* strains TA1535 (base-pair mutant) or TA1538 (frame shift mutant) after liver microsomal activation; however, no details of the concentrations tested were given. Although tetrachlorocyclopentadiene is mutagenic in these systems, probably through metabolic conversion to the dienone, it appears that the chlorine atoms at the C-1 position of HEX hindered metabolic oxidation to the corresponding acylating dienone (Greim et al., 1977).

A study conducted by Industrial Bio-Test Laboratories (IBT, 1977) also suggests that HEX is not mutagenic in S. typhimurium. Both HEX and its vapors were tested with and without metabolic activation. The vapor test was done in desiccators with only the TA-100 strain of S. typhimurium. It is not clear from the presented data of the test with the vapors that sufficient amounts of HEX or adequate times of exposure were used. Exposure times of 30, 60 or 120 minutes were studied. Longer exposures in the presence of the HEX vapors may be necessary for observation of a potential mutagenic effect.

At concentrations of up to $1.25 \times 10^{-3}$ μg/mℓ in the presence of an S-9 liver activating system, HEX was not mutagenic in the mouse lymphoma mutation assay. Mutagenicity could not be evaluated at higher concentrations because of the cytotoxicity of HEX (Litton Bionetics, Inc., 1978a). This assay uses L5178Y cells that are heterozygous for thymidine kinase (TK+/-) and are bromodeoxyuridine (BUdR) sensitive. The mutation is scored by cloning with BUdR in the absence of thymidine. HEX is highly toxic to these cells, particularly in the absence of activating system (at $4 \times 10^{-5}$ μℓ/mℓ) and a positive control, dimethylnitrosamine, was mutagenic at 0.5 μℓ/mℓ.

Williams (1978) found that HEX ($10^{-6}$ M) was inactive in the liver epithelial culture hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus/mutation assay. At $10^{-5}$ M it also failed to stimulate DNA repair synthesis in hepatocyte primary cultures. Negative results were also obtained in an additional unscheduled DNA synthesis assay (Brat, 1983).

Two recent studies provided by NTP (Juodeika, 1983) also did not demonstrate the mutagenicity of HEX. In S. typhimurium strains TA98, TA100, TA1535 and TA1537, levels of up to 3.3 μg/plate were not mutagenic without activation and levels of up to 100.0 μg/plate were not mutagenic after microsomal activation. Higher levels could not be tested because of excessive killing of the bacteria. In the Drosophila sex-linked recessive lethal test, HEX was not mutagenic. The doses used in this study were 40 ppm by feeding for 3 days or a single injection of 2000 ppm.

HEX has also been assayed in the mouse dominant lethal test (Litton Bionetics, Inc., 1978b). In this assay, 0.1, 0.3 or 1.0 mg/kg HEX was administered by gavage to 10 male CD-1 mice for 5 days and these mice were then mated throughout spermatogenesis (7 weeks). This test determines whether the compound induces lethal genetic damage to the germ cells of males. There was no evidence of dominant lethal activity at any dose level by any parameter; e.g., fertility index, implantations/pregnancy, average resorptions/pregnancy.

**7.3.2. Summary.** The available evidence suggests that HEX is not a mutagen. Negative mutagenicity results were obtained in bacteria, liver epithelial cells, Drosophila, mouse lymphoma cells and in the mouse dominant lethal test. Furthermore, HEX did not induce unscheduled DNA synthesis in rat hepatocytes.

## 7.4. CARCINOGENICITY

**7.4.1. In Vivo Carcinogenicity.** Bioassays of HEX for possible carcinogenicity have not been conducted. However, NTP has scheduled HEX for carcinogenicity testing by the inhalation route in rats and mice (Abdo, 1983).

**7.4.2. In Vitro Carcinogenicity.** The ability of HEX to induce morphologic transformation of BALB/3T3 cells in vitro has been studied by Litton Bionetics, Inc. (1977). The procedure employed by the investigators was similar to that of Kakunaga (1973). Evaluation of the carcinogenic activity was based on the following criteria:

> The endpoint of carcinogenic activity is determined by the presence of fibroblastic-like colonies which are altered morphologically in comparison to the cells observed in normal cultures. These (transformed) cells grow in criss-cross, randomly oriented fashion with overlapping at the periphery of the colony. The colony exhibits dense piling up of cells. On staining the foci are deeply stained and the cells are basophilic in character and variable in size. These changes are not observed in normal cultures, which stain uniformly.

Assays were performed at levels of 0.0, 0.01, 0.02, 0.039, 0.078 and 0.156 μℓ/mℓ. The cultures were exposed for 48 hours followed by an incubation period of 3-4 weeks. The cultures were observed daily. The selection of test doses was based on previous cytotoxicity tests using a wide range of HEX concentrations. The doses selected allowed an 80-100% survival of cells as compared with solvent negative controls. This high survival rate permitted an evaluation of _in_ _vitro_ malignant transformation in cultures treated with HEX as compared with the solvent controls. 3-Methylcholanthrene at a dose level of 3 μg/mℓ was used as a positive control. Results indicated that HEX was not responsible for any significant carcinogenic activity.

**7.4.3. Summary.** HEX has not been demonstated to be a carcinogen _in_ _vitro_ in transformation assays using BALB/3T3 cells. _In_ _vivo_ bioassays have not been conducted; however, an inhalation bioassay has been scheduled by the National Toxicology Program.

## 7.5. TERATOGENIC AND REPRODUCTIVE EFFECTS

**7.5.1. Teratogenicity.** The teratogenic potential of HEX was evaluated in pregnant Charles River CD-1 rats that were administered HEX (98.25%) in corn oil, by gastic intubation, at dose levels of 3, 10 and 30 mg/kg/day from days 6 through 15 of gestation. A control group received the vehicle (corn oil) at a dose volume of 10 mℓ/kg/day. Survival was 100%, and there was no difference in mean maternal body weight gain between dosed groups and controls. There were no differences in the mean number of implantations, corpora lutea, live fetuses, mean fetal body weights or male/female sex ratios among any of the groups, and there were no statistical differences in malformation or developmental variations compared with the controls when external, soft tissue and skeletal examinations were performed (IRDC, 1978).

Murray et al. (1980) evaluated the teratogenic potential of HEX (98%) in CF-1 mice and New Zealand white rabbits. Mice were dosed at 0, 5, 25 or 75 mg/day HEX by gavage from days 6-15 of gestation while rabbits received the same dose from days 6-18 of gestation. The fertility of both the treated mice and rabbits was not significantly different from the control groups. In the mice, no evidence of maternal toxicity, embryotoxicity or teratogenic effects was observed. A total of 249-374 fetuses (22-33 litters) were examined in each dose group.

In rabbits, maternal toxicity was noted at 75 mg/day (diarrhea, weight loss and mortality), but there was no evidence of maternal toxicity at the lower levels. There were no embryotoxic effects at any dose level. Although there was an increase in the proportion of fetuses with 13 ribs at 75 mg/day over controls, this was considered a minor skeletal variation, and the authors concluded that HEX was not teratogenic at the levels tested.

Studies on the teratogenic potential of inhaled HEX were not located in the review of the scientific literature.

7.5.2. **Reproductive Effects.** No data were located that addressed the reproductive effects of HEX.

7.5.3. **Summary.** HEX has been tested for teratogenic potential by oral gavage in rats, mice and rabbits. No maternal toxicity or teratogenic effects were noted in rats or mice when HEX was administered on days 6 through 15 of gestation at doses of up to 25 and 75 mg/day, respectively. Rabbits exhibited maternal toxicity when HEX was administered at 75 mg/day from days 6 through 18 of gestation and an increase in fetuses with 13 ribs was also noted at this dose level. The latter was considered to be a minor skeletal variation by the authors. No maternal toxicity or fetal abnormalities were noted in rabbits at lower doses. HEX therefore does not appear to be teratogenic by oral gavage in the species and at the doses tested. HEX was not tested for teratogenicity following inhalation exposures.

## 7.6. HUMAN EXPOSURE AND HEALTH EFFECTS

7.6.1. **Human Exposure.** According to a recent NIOSH estimate, 1427 workers are occupationally exposed to HEX (NIOSH, 1980). Velsicol officials estimate that approximately 157 employees are potentially exposed to HEX in their production facilities. A summary of monitoring results is presented in Tables 7-7 and 7-8 for the Velsicol Memphis and Marshall plants, respectively. In addition, acute human exposure has been reported in homes near waste sites where HEX has been disposed (S. Clark et al., 1982; Elia et al., 1983).

7.6.2. **Health Effects.** Very little detailed information is available concerning the effects of HEX exposure on humans. The odor threshold has been stated to be 0.00017 ppm, however, there has been great individual variation. According to the data provided in a study completed by A.D. Little for Occidental Chemical Corporation, the 100% panel recognition concentration was 0.0017 mg/m³ (0.00017 ppm v/v) (Levins, 1980). The study design and methodology was not given. According to the Material Safety Data

## TABLE 7-7

### Memphis HEX Monitoring Summary
### (Velsicol Chemical Corporation, April 6, 1982)*

| Unit | Description | No. of Samples | Average Duration (minutes) | Range of Sample Concentrations (ppm) | Average TWA (ppm) |
|---|---|---|---|---|---|
| HEX | Process Operator | 2 | 445 | 0.009 - 0.011 | 0.009 |
| HEX | No. 1 Operator | 5 | 432 | 0.006 - 0.033 | 0.015 |
| HEX | No. 2 Process Operator | 5 | 418 | 0.006 - 0.029 | 0.014 |
| HEX | No. 2 Cyclo Operator | 5 | 417 | 0.001 - 0.048 | 0.017 |
| HEX | No. 2 Chlorine Operator | 6 | 415 | 0.004 - 0.016 | 0.008 |
| HEX | Environmental Operator | 6 | 436 | 0.004 - 0.161 | 0.035 |
|  | a) HEX Bottoms Drumming | 1 | 50 | 0.016 | -- |
| HEX | Area Sample Control Room | 12 | 476 | 0.002 - 0.018 | 0.009 |
| HEX | Brinks Filter Cleaning (maintenance personnel) | 2 | 387 | 0.004 - 0.006 | 0.005 |
| Formulations | HEX Drummers | 4 | 407 | 0.002 - 2.0337 | 0.010 |
| Materials Handling | HEX Railroad Tank Car Unloading | 1 | 279 | 0.013 | 0.008 |
| Endrin | R2 Filter Operator | 1 | 281 | 0.003 | -- |
| Endrin | R1 Operator | 1 | 334 | 0.002 | -- |
| C.A. | No. 1 Operator | 2 | 437 | 0.0077 - 0.0102 | 0.008 |
| C.A. | No. 2 Operator D34 | 2 | 440 | 0.0107 - 0.0198 | 0.014 |
| C.A. | No. 2 Operator R6 | 2 | 437 | 0.0065 - 0.0169 | 0.011 |

TABLE 7-7 ([illegible])

| Unit | Description | No. of Samples | Average Duration (minutes) | Range of Sample Concentrations (ppm) | Average TWA (ppm) |
|---|---|---|---|---|---|
| C.A. | Packaging Operator | 1 | 396 | 0.035 | 0.031 |
| C.A. | Area Sample - control room | 3 | 475 | 0.0003 - 0.0014 | 0.001 |
| Heptachlor | No. 1 Operator | 2 | 407 | 0.007 - 0.009 | 0.007 |
| Heptachlor | No. 2 Operator Catalyst | 2 | 415 | 0.006 - 0.009 | 0.007 |
| Heptachlor | 237 Operator | 2 | 392 | 0.006 - 0.019 | 0.011 |
| Heptachlor | Utility Operator | 1 | 363 | 0.006 | 0.005 |
| Heptachlor | Cleaning Sparkler Filter | 3 | 44 | 0.002 - 0.005 | 0.0003 |
|  | a) Ceiling Sample | 1 | 15 | 0.006 | -- |

*Source: Levin 1982a

ppm = parts of HEX per million parts of air by volume

TWA = 8-hour time-weighted average. The TWA calculation was made assuming that the only chemical exposure was during the sampling period.

C.A. = chlorendic anhydride.

NOTE: The employee monitoring (indicated job function) results are reported without regard to respirator use. For operators where HEX exposure is possible, respirators are required and are worn.

TABLE 7-8

Marshall HEX Monitoring Summary
(Velsicol Chemical Corporation, April 6, 1982)*

| Unit | Description | No. of Samples | Average Duration (minutes) | Range of Sample Concentrations (ppm) | Average TWA (ppm) |
|---|---|---|---|---|---|
| Chlordane | No. 1 Operator | 8 | 451 | 0.0091 - 0.0316 | 0.017 |
| Chlordane | No. 2 Operator | 8 | 455 | 0.0080 - 0.0195 | 0.013 |
| Chlordane | No. 3 Operator | 8 | 451 | 0.0002 - 0.0325 | 0.014 |
| Chlordane | Area sample - North Control Room | 13 | 433 | 0.0002 - 0.0254 | 0.016 |
| Chlordane | HEX Filter Changing | 1 | 15 | 0.1322 | -- |
| Chlordane | Waste Handling HEX Mud Drumming | 6 | 307 | 0.0006 - 0.0606 | 0.020 |
| Chlordane | a) Ceiling Sample | 2 | 15 | 0.0005 - 0.0061 | -- |
|  | b) Loading HEX Waste Truck - Ceiling Sample | 2 | 15 | 0.1199 - 0.2325 | -- |
|  | c) Sump Pit Dumping - Ceiling Sample | 2 | 15 | 0.0333 - 0.1129 | -- |

*Source: Levin 1982a

ppm = parts of HEX per million parts of air by volume

TWA = 8-hour time-weighted average. The TWA calculation was made assuming that the only chemical exposure was during the sampling period.

NOTE: The employee monitoring (indicated job function) results are reported without regard to respirator use. For operators where HEX exposure is possible, respirators are required and are worn.

Sheet prepared by Hooker Chemical Corporation (1979) and based on animal studies, HEX vapors are very irritating to all mucous membranes, causing tearing, sneezing and salivation; skin contact can cause blisters and burns; inhalation of vapors or mists can result in the secretion of excess fluid in the lungs; and inhalation or ingestion may cause nausea, vomiting, diarrhea, lethargy, respiratory impairment and injury to the liver or kidneys.

7.6.2.1. EFFECTS FOLLOWING INCIDENTS OF ACUTE EXPOSURE -- Treon et al. (1955) reported that members of a group conducting toxicity tests developed headaches when they were accidentally exposed to unknown concentrations of HEX, which had escaped into the room when an aerated exposure chamber was opened.

A well-documented incident of acute human exposure to HEX occurred in March 1977 at the Morris Forman Wastewater Treatment Plant in Louisville, Ky. The incident has been described and reviewed in several papers (Kominsky et al., 1980; Wilson et al., 1978; Morse et al., 1979). The complete details of the original NIOSH Hazard Evaluation and Technical Assistance Report Number TA-77-39 (Kominsky et al., 1978) are available from the National Technical Information Service (NTIS).

In 1977, the Louisville treatment facility was contaminated with ~6 tons of HEX and OCCP, a waste byproduct of HEX manufacture (Morse et al., 1979). The contamination was traced to one large sewer line that passed through several populated areas. Concentrations of HEX detected in the sewage water at the plant ranged as high as 1000 ppm, and levels in the sewer line ranged up to 100 ppm. Air samples from the sewer line showed HEX concentrations as high as 400 ppb. Although airborne concentrations of HEX at the time of the exposure were unknown, airborne concentrations in the primary treatment areas (screen and grit chambers) ranged between 270 and 970 ppb 4 days after the plant had closed. (The TWA for HEX was 10 ppb in 1977.) During the cleanup of the contamination, workers using steam attempted to remove an odoriferous and sticky substance from the bar screens and grit collection system. This produced a blue haze which permeated the primary treatment area. Airborne HEX concentration of the blue haze generated by the cleanup procedures was reported to be 19.2 ppm (Kominsky et al., 1980).

Both the Center for Disease Control (CDC) and NIOSH sent representatives to the plant, with each group developing questionnaires seeking information on the type and duration of symptoms (Morse, et al., 1979; Kominsky, et al., 1980). A total of 193 employees were identified as those potentially exposed for 2 or more days during the 2 weeks before the plant was closed (Morse et al., 1979). A questionnaire was sent to each of these workers and 145 (75%) responded. Workers with complaints of mucous membrane irritation were given a physical examination, and blood and urine samples were collected for clinical screening by an independent laboratory. Data were also collected on the exposure levels and symptoms in several individual cases of acute exposure to the chemical vapors.

Results of the CDC and NIOSH questionnaires showed that the odor of HEX was detected before the onset of symptoms by 94% of the workers. The most common symptoms reported were eye irritation (59%), headaches (45%) and throat irritation (27%) (Table 7-9). Of the 41 workers physically examined, 6 had physical signs of eye irritation (i.e., tearing or redness) and 5 had signs of skin irritation. Laboratory analyses of blood and urine specimens from these workers showed elevations of lactic dehydrogenase (LDH) in 27% and proteinuria in 15%. However, no clinical abnormalities were reported by the plant physician, the local hospital, or by the independent laboratory 3 weeks later (Morse et al., 1978, 1979).

## TABLE 7-9

### Symptoms of 145 Wastewater Treatment Plant Employees Exposed to HEX (Louisville, KY, March 1977)*

| Symptom | No. of Employees with Symptom | Percent of Employees with Symptom |
|---|---|---|
| Eye irritation | 86 | 59 |
| Headache | 65 | 45 |
| Throat irritation | 39 | 27 |
| Nausea | 31 | 21 |
| Skin irritation | 29 | 20 |
| Cough | 28 | 19 |
| Chest pain | 28 | 19 |
| Difficult breathing | 23 | 16 |
| Nervousness | 21 | 14 |
| Abdominal cramps | 17 | 12 |
| Decreased appetite | 13 | 9 |
| Decreased memory | 6 | 4 |
| Increased saliva | 6 | 4 |

*Source: Morse et al., 1978

While there was difficulty in measuring the amount of exposure by the plant workers, over half of the cleanup crew was monitored. Laboratory tests showed no significant abnormalities, however, several minimal-to-mild abnormalities did appear in liver function tests (Kominsky et al., 1980). These abnormalities are listed in Table 7-10. All of these affected persons also had physical signs of mucous membrane irritation. In addition, more detailed correlation of acute exposure level data to symptomatology was reported for 9 adults (Kominsky et al., 1980). These data are reviewed in Table 7-11. The exposure levels could not be estimated accurately because of prior exposure or because the worker had used protective equipment.

A questionnaire was also given to a selected sample of residents of a 48-block area surrounding the contaminated sewer line. A total of 212 occupants were surveyed. Very few residents noted an unusual odor (3.8%). The most prevalent symptoms were stomachaches (5.2%), burning or watering eyes (4.7%) and headaches (4.7%). There was no association between symptom rates and the distance of households from the contaminated sewer line. The authors stated that no significant ambient air concentrations of HEX were found in these areas (Kominsky et al., 1978). The same types and frequency of symptoms reported by workers to be associated with HEX exposure were reported by residents in the survey which led the authors to suggest that these symptoms were unrelated to HEX exposure (Morse et al., 1978).

Several papers have documented another similar incident in Hardeman County, TN. (S. Clark et al., 1982; Meyer, 1983; Elia et al., 1983). While conducting a serioepidemiologic study of the health risks from bacteria and viruses associated with the treatment of municipal wastewater, potential human exposure to organic chemicals emitted from the wastewater being treated at one of the plants in the study was recognized (Elia et al.,

TABLE 7-10

Abnormalities for 18 of 97 Cleanup Workers at the Morris Forman Treatment Plant[a]

| | | Abnormal Results | |
|---|---|---|---|
| Laboratory Test | Normal Range | Range | No.[b] |
| Serum Glutamate-Oxalacetate Transaminase | 7-40 mU/mℓ | 40-49 | 5 |
| | | 50-59 | 1 |
| | | 60-69 | 4 |
| | | 70-79 | 0 |
| | | 80-89 | 1 |
| | | 90-99 | 1 |
| Serum Alkaline Phosphatase | 30-100 mU/mℓ | 100-109 | 3 |
| | | 110-119 | 1 |
| | | 120-129 | 1 |
| Serum Total Bilirubin | 0.15-10 mg/% | 1.0-1.9 | 1[c] |
| Serum Lactate Dehydrogenase | 100-225 mU/mℓ | 230-239 | 1 |

[a] Kominsky et al., 1980

[b] For individuals with more than one serial blood test, only the most abnormal result is tabulated.

[c] Associated with serum glutamate-oxalacetate transaminase of 66

U = Units of enzyme activity

### TABLE 7-11

### Overview of Individual Exposure - Symptomatology Correlations at the Morris Forman Treatment Plant[a]

| Case No. | Estimated Airborne Exposure | Immediate Symptoms | Persistence of Symptoms | Laboratory Abnormalities |
|---|---|---|---|---|
| 1 | 19,200 ppb HCCPD and 650 ppb OCCP for several seconds (No protective equipment) | Lacrimation; skin irritation on face and neck; dyspnea and chest discomfort; nausea (several minutes later) | 1.5 hrs. post-exposure: Fatigue; erythema of exposed skin; eye irritation subsided in 1 day; chest discomfort persisted several days. | Lab work 4 days post exposure was normal[b] |
| 2,3,4 | 7083 ppb HCCPD and 446 ppb OCCP for several seconds. (Half-face respirator) | Lacrimation; irritation of exposed skin | Asymptomatic at 2 hours, except for soreness around eyes | Lab work 7 days post exposure was normal on one worker[b] |
| 5,6 | 40-52 ppb HCCPD and 9-21 ppb OCCP (Half-face respirator) | Slight eye irritation | No residual after cessation of exposure | Normal 7 days later on one |
| 7,8 | Exact exposure unknown (Half-face respirator) | Slight skin irritation | Faces felt "puffy" and "windburned" for 1-2 days after exposure. This was noted also by friends and family. No residual skin lesions. | None available |
| 9 | 980 ppb HCCPD for 15 minutes; OCCP not measured (No protective equipment) | Irritated eyes<br>Nasal irritation and sinus congestion after 2 weeks of intermittent exposures | Eyes felt "dry and irritated" for 2-3 days after exposure. Nasal irritation ceased within 1-2 days of cessation of exposure. | None available |

[a] Source: Kominsky, et al., 1980

[b] Laboratory work was same as done on cleanup crew

1983). In 1978, workers at the treatment plant began complaining of acute symptoms similar to those found in the Louisville plant. Air and wastewater monitoring was started, analysis of urine specimens, analysis of blood and liver function tests, and an illness symptom questionnaire were used to collect data. In the original study design, workers were compared to a control group from another Memphis treatment plant which does not receive wastes from the pesticide manufacturing plant. In a later survey, workers at two other municipal facilities were used for comparison. In the analysis of the various monitoring tests, S. Clark et al. (1982) found no statistical difference in urine samples from both of the Memphis treatment facilities. In the liver function tests, there were no statistically significant differences among the values obtained for all survey groups.

About the time the wastewater treatment plant study was being performed, residents of Hardeman County in the general area of the plant began to complain of foul odors and bad taste in their well water and asked for an investigation (Meyer, 1983). In this area lies a 200 acre chemical land dump which was operated from 1964-1972. In 1978, the U.S. Geologic Survey (Sprinkle, 1978; Rima, 1979) confirmed the contamination of wells. However, HEX was not detected in any samples. Urine surveys and liver function analyses were conducted. Utilizing an unexposed group (those not exposed to the treatment facility or the contaminated water), a comparison of various liver enzymes was done (Table 7-12). The situation at the Memphis treatment facility is the only known existing case of essentially continuous low-level chronic exposures with intermittent higher acute exposures, especially during an accidental discharge from the nearby pesticide manufacturing facility (Elia, 1983).

## TABLE 7-12

Hepatic Profile Comparison of Hardeman County: Exposed Group (November 1978) and Control Group[a]

| Parameter[b] | | Results | | Significance of Difference (t test) |
|---|---|---|---|---|
| | | November 1978 Exposed Group | Control Group | |
| Alkaline phosphatase (32-72 mU/mℓ age 21, 25-150 mU/mℓ age 21) | Mean[c] | 88.1 | 61.5 | 0.016 |
| | Range | 34-360 | 31-220 | |
| | No. above normal/ total tested | 17/36 | 8/56 | |
| Serum gamma glutamic transaminase (SGGT) (5-29 mU/mℓ) | Mean[c] | 9.47 | 11.56 | 0.430 |
| | Range | 2-54 | 4-56 | |
| | No. above normal/ total tested | 3/36 | 3/56 | |
| Albumin (3.5-5.0 g/dℓ) | Mean[c] | 4.35 | 4.93 | 0.0001 |
| | Range | 3.9-4.8 | 4.2-6.2 | |
| | No. above normal/ total tested | 0/36 | 0/57 | |
| Total bilirubin (0.1-1.1 mg/dℓ) | Mean[c] | 0.240 | 0.51 | 0.0001 |
| | Range | 0.1-0.8 | 0.2-1.7 | |
| | No. above normal/ total tested | 0/31 | 4/52 | |
| Serum glutamic pyruvic transaminase (SGOT) (8-22 mU/mℓ) | Mean[c] | 19.5 | 16.08 | 0.001 |
| | Range | 12-36 | 9-140 | |
| | No. above normal/ total tested | 11/36 | 7/56 | |

[a] Source: Meyer, 1983

[b] Normal range indicated in parentheses

[c] Geometric mean

U = Units of enzyme activity

The Hardeman County studies have been the subject of much scrutiny and court litigation. At the time of this publication, there has not been any legal decision rendered. Because of questions concerning the various study designs used in the studies, very few conclusions can be reached until further monitoring can be completed. However, these two incidents illustrate the possibility of acute exposure at waste treatment facilities receiving industrial waste.

7.6.2.2. EPIDEMIOLOGIC STUDIES -- Mortality studies have been conducted on the workers involved in the production of HEX or formulation of HEX products. The Shindell report (1980) was a cohort study of workers employed at the Velsicol Chemical Corporation plant at Marshall, Illinois between 1946 and 1979. The purpose was to evaluate the vital status of all former and current employees (>3 months) who were present during the manufacture of chlordane. In preparing the cohort, the authors noted the difficulties in tracing some of the employees. In the final cohort of 783 individuals, 97.4% of the employees were located and their vital status included in the study. The analysis showed no significant differences in mortality rates between these employees and the U.S. population. The observed deaths for all causes, including heart disease and cancer, were fewer than the calculated expected deaths among members of the U.S. population (Shindell and Associates, 1980).

Wang and MacMahon (1979) conducted a study on a group of 1403 males employed at the Marshall and Memphis plants for >3 months. There were 113 observed deaths compared with 157 expected, yielding a standardized mortality ratio (SMR) of 72, not remarkable for an employed population. The 2 highest SMRs were 134 for lung cancer and 183 for cerebrovascular disease, but only the latter was statistically significant ($\underline{p}<0.05$). The authors suggested that these effects were unrelated to exposure because the deaths showed no consistent pattern with duration of employment or with duration of follow-up.

Shindell and Associates (1981) completed an epidemiologic study for Velsicol. The study group consisted of over 1000 employees (93% of the cohort) of the Memphis, Tn plant for the years 1952-1979, coinciding with the manufacture of heptachlor. Again, the researchers found no significant difference in mortality between the control and exposure groups and fewer deaths in the study group. The investigators report that there was no excess mortality by job function.

Buncher et al. (1980) studied the mortality of workers at a chemical plant that produced HEX. The investigators reviewed personnel who worked for at least 90 days between October 1, 1953 and December 31, 1974. There were 341 workers (287 male and 54 female) who fit the criteria. Health status was ascertained through 1978 and expected numbers of deaths were calculated based upon the U.S. population and specific for sex, age and calendar year. The SMR was 69 which showed the workers to be healthier than the general population. Deaths caused by specific cancers, all cancers, disease of the circulatory and digestive systems were fewer than the expected numbers. The authors noted that the time since initial exposure, at the most 25 years, reduced the power of the study to detect cancers which may have a 10-40 year latent period.

7.6.3. **Summary.** While there is human experience with respect to mortality, there is only limited information on the morbidity results in those exposed to HEX. Acute inhalation produces a high prevalence of headaches and severe irritation of the eyes, nose, throat and lungs. Dermal contact can cause severe burns. Epidemiologic studies have generally shown no significant differences in mortality between workers exposed to HEX in the workplace and the general population. Although, a significant excess of deaths from cerebrovascular disease was reported in one study, the deaths showed no consistent pattern with duration of employment or follow-up.

Current human exposure is limited to improper handling and disposal and proximity to either manufacturing sites utilizing HEX or disposal sites. No other chronic human health effects data from HEX exposure have been located in the literature.

# 8. OVERVIEW

## 8.1. EFFECTS OF MAJOR CONCERN

Although minimal quantitative information is available on the effects of HEX on humans, transient exposure to HEX vapor has been found to cause irritation to the eyes, nose and throat, as well as headaches. The levels of exposure causing these effects are not well defined but they are at a level close to the odor threshold, which varies individually and may be as low as 0.00017 ppm (0.0017 mg/m$^3$). There is no information on the long-term effects of a single exposure or of subchronic exposure. There is no information available on the carcinogenicity of HEX. *In vitro* mutagenicity or transformation tests were negative. The *in vivo* mouse dominant lethal assay was negative at the tested levels. HEX has not been shown to be teratogenic in studies examining three species.

Considering all of the above facts, the major concerns of HEX exposure are the toxic effects on the respiratory system when HEX is inhaled. Although the chronic toxicity data are presently limited, the systemic toxic effects of HEX inhalation have been demonstrated after acute and subchronic exposure, suggesting that chronic inhalation exposure to low doses of HEX may have adverse effects.

**8.1.1. Principal Effects and Target Organs.** Repeated exposure of several animal species to levels of HEX vapor in the 0.1-0.2 ppm range has been found to cause pulmonary degenerative changes (Treon et al., 1955; Rand et al., 1982a,b; S. Clark et al., 1982). Treon et al. (1955) reported mild degenerative changes in the kidneys, liver, brain, heart and adrenal glands. Rand et al. (1982), however, did not confirm this and suggested that the changes found by Treon et al. (1955) were caused by impurities in the preparation of HEX. Acute exposure by oral and dermal routes also cause effects on the respiratory system (Kommineni, 1978; SRI, 1980a). Death from acute exposure by any tested route appears to be associated with respiratory failure (Lawrence and Dorough, 1981).

There are insufficient data to identify clearly the site most sensitive to prolonged, repeated exposure to HEX. However, researchers found in comparing routes of administraton that regardless which route was used, damage to the lungs occurred (Lawrence and Dorough, 1982). When HEX is administered orally to animals, the kidneys may be the most sensitive site, since subchronic dosing of rats and mice was found to cause nephrosis especially in females (SRI, 1981a,b). Although the oral route may not be significant in human exposure, the fact that the kidneys are a possible target organ in subchronic exposure indicates that low-level, prolonged systemic exposure from any ambient route may affect the kidneys. The liver has also been an affected organ as seen in many of the laboratory studies.

**8.1.2. Animal Toxicity Studies Most Useful for Hazard Assessments.** The studies most useful for prediction of hazards are those that use a variety of dose levels, a variety of species, adequate sample sizes, and display the full range of effect severity, from no effects through mortality. The major quantitative goal is to estimate the threshold level for adverse effects, i.e., the level at or above which adverse effects are observed. In this regard, the most appropriate studies are those presenting no-observed-effect levels (NOEL), no-observed-adverse-effect levels (NOAEL) and adverse-effect levels (AEL), i.e., those dose rates which bracket the threshold level (Tables 8-1 and 8-2). Dose rates labeled "EL" (for "effect level") are associated with effects which may or may not be adverse, based upon the data presented by the researchers. Because dosing regimens varied among studies, a time-weighted-average (TWA) daily exposure level has been calculated to

## TABLE 8-1

### Oral Toxicity Data for Threshold Estimates

| Animal | Exposure Duration (days) | Exposure Level[a] | Effect Severity[b] | Reference |
|---|---|---|---|---|
| Rat | 10 | 10 mg/kg | NOEL | IRDC, 1978 |
| | | 30 mg/kg | EL | IRDC, 1978 |
| | | 100 mg/kg | AEL | IRDC, 1978 |
| Rat | 12 | 25 mg/kg | NOAEL | SRI, 1980b |
| | | 50 mg/kg | AEL | SRI, 1980b |
| Mouse | 12 | 50 mg/kg | EL | SRI, 1980a |
| | | 100 mg/kg | AEL | SRI, 1980a |
| Rat | 91 | 7 mg/kg | NOAEL | SRI, 1981a |
| | | 14 mg/kg | EL | SRI, 1981a |
| | | 27 mg/kg | AEL | SRI, 1981a |
| Mouse | 91 | 14 mg/kg | NOAEL | SRI, 1981b |
| | | 27 mg/kg | EL | SRI, 1981b |
| | | 54 mg/kg | AEL | SRI, 1981b |
| Rat | 216 | 0.2 mg/kg | NOEL | Naishtein and Lisovskaya, 1965 |
| | | 2.0 mg/kg | EL | |

[a] Time-weighted-average daily exposure levels

[b] Definitions:
NOEL - No-observed-effect level
NOAEL - No-observed-adverse-effect level
EL - Effect level
AEL - Adverse effect level

## TABLE 8-2

## Inhalation Toxicity Data for Threshold Estimates

| Animal | Exposure Duration (days) | Exposure Level[a] | Effect Severity[b] | Reference |
|---|---|---|---|---|
| Rat | 14 | 0.004 ppm | NOAEL | Rand et al., 1982a |
| | | 0.020 ppm | EL | Rand et al., 1982a |
| | | 0.089 ppm | AEL | Rand et al., 1982a |
| Rat, guinea pig | 42 | 0.069 ppm | AEL | Treon et al., 1955 |
| Rat | 90 | 0.002 ppm | NOAEL | Rand et al., 1982a |
| | | 0.009 ppm | NOAEL | Rand et al., 1982a |
| | | 0.036 ppm | EL | Rand et al., 1982a |
| Monkey | 90 | 0.002 ppm | NOAEL | Rand et al., 1982a |
| | | 0.009 ppm | NOAEL | Rand et al., 1982a |
| | | 0.036 ppm | NOAEL | Rand et al., 1982a |
| Rat | 210 | 0.009 ppm | NOEL | Clark et al., 1982 |
| | | 0.018 ppm | EL | Clark et al., 1982 |
| | | 0.089 ppm | AEL | Clark et al., 1982 |
| Rat, rabbit, guinea pig | 216 | 0.031 ppm | AEL | Treon et al., 1955 |

[a] Time-weighted-average daily exposure levels

[b] Definitions:
NOEL - No-observed-effect level
NOAEL - No-observed-adverse-effect level
EL - Effect level
AEL - Adverse effect level

use as a comparison. This value assumes a continuous 24-hour ambient exposure. For example, at the highest actual dose level (0.5 ppm) in the Rand et al. (1982a,b) studies, the equation would be as follows:

$$\text{TWA level} = 0.5 \text{ ppm} \times \frac{5 \text{ days}}{7 \text{ days}} \times \frac{6 \text{ hours}}{24 \text{ hours}} = 0.089 \text{ ppm.}$$

Toxicity from inhalation of HEX appears to be more severe than that of oral or dermal exposure and may be the cause of so few inhalation studies showing minor effects. Rand et al. (1982a,b) used sufficiently low concentrations in a 14-day study on rats and in a 90-day study on rats and monkeys to elicit effect levels. Clark and researchers (D. Clark et al., 1982) found that rat groups (18 males and 18 females per group) exposed to HEX at 0.05 ppm (0.009 ppm daily TWA) for 30 weeks showed no effects. However, Rand et al. (1982a) found their animals had demonstrated some effects at the same level (0.009 ppm daily TWA) in only 90 days. Treon et al. (1955) exposed their animals for 216 days and found adverse effects at 0.03 ppm daily TWA.

Short-term oral studies by IRDC (1978) and SRI (1980a,b) provide information on toxicity to rats and mice, although the study sizes were small (5 and 10 animals per dose group, respectively). The 90-day study by SRI (1981a,b) on rats and mice is the only short-term oral study providing no-adverse-effect levels, and the Naishtein and Lisovskaya (1965) 6-month study on rats is the only long-term data set giving no-effect levels. These three studies had marginally adequate sample sizes.

The remaining studies detailed in Chapter 7, and those listed in the toxicity table in the Appendix, provide information on more severe effects that can be used to show consistency with the threshold estimates. By themselves, however, they cannot be used to estimate a threshold since none adequately describes the shape of the dose-response severity relationship. For example, dose rates associated with NOFELs (no-observed-frank-effect-levels) indicate that no significant change in frank effects was attributed to the exposure. Milder effects were not investigated, so that the NOFEL could dramatically overestimate the threshold.

## 8.2. FACTORS INFLUENCING HEALTH HAZARD ASSESSMENT

**8.2.1. Exposure.** Data are available regarding the potential human exposure to HEX. It appears that any significant exposure would be the result of improper disposal or accidental spill. Limited data were presented for the air and water levels of HEX in these incidents. Emissions data, from which atmospheric exposure estimates could be derived, have been sent to the U.S. EPA, but are considered confidential business information (CBI) and are not available in this report. No HEX residue was detected in fish taken from the waters near a production plant in Memphis in 1982. No information was available regarding HEX contamination of other foods. Although occupational exposure is expected to be minimal, the long-term health effects of continuous low-level exposure and/or intermittent acute exposure in man are not known. Waste handlers and sewage treatment workers have been shown to be occupations at risk.

**8.2.2. Lowest-Observed-Effect Level.** Both single dose and short-term range-finding inhalation studies (7 hours/day) by Rand et al. (1982a) demonstrated "a steep dose response effect of HEX exposure with a threshold of toxicity in rats between 0.11 and 0.5 ppm." This observation is based on severe irritation of the lungs, consequent inflammation, and impaired respiratory function in rats. The TWA daily exposure levels, from the NOAEL to the AEL, give a range between 0.004-0.089 ppm. Subchronic exposure (~90 days) to rats and monkeys (Rand et al., 1982a) indicate a threshold range between 0.002-0.036 ppm based on TWA daily dose rates. D. Clark et al. (1982) exposed rats for 30 weeks and found adverse effects in the 0.089 ppm TWA range with no adverse effects at 0.009 ppm TWA. However, Treon et al. (1955) exposed rabbits, rats and guinea pigs to a TWA level of 0.031 ppm for 216 days and caused moderate adverse effects, so the lifetime experimental threshold is likely to be somewhat less. No lifetime data exist for determining NOELs or NOAELs.

As expected, the toxicity from HEX inhalation seems highly dependent upon the dosing rate and regimen. In several studies, a dose change of less than one order of magnitude separated minor effects from increased mortality. This pattern was observed for acute studies through chronic studies. In the previous comparison of threshold levels, the difference between effects and no-observed-adverse effects depends to a large degree on the researchers' documentation and detailed discussion of the observed effects shown by HEX exposure. With the narrow range between these dose levels, the determination of exact separations between effect levels and adverse effect levels is limited by the published data.

The short-term oral studies (IRDC, 1978; SRI, 1980a,b) indicate a lowest effect range for daily exposure to be 25-100 mg HEX/kg bw, based on rat and mouse data. Subchronic oral studies (SRI, 1981a,b) suggest a lowest effect range of 7-54 mg HEX/kg bw/day based on TWA dose rates used with rats and mice. The rats responded at lower doses than did the mice, but the metabolic similarities to man are not sufficiently well understood to allow choice of a best animal model. Chronic oral HEX exposure to 0.2-2.0 mg/kg showed no adverse effects (Naishtein and Lisovskaya, 1965).

**8.2.3. Carcinogenicity.** There are no animal bioassay data indicating that HEX is carcinogenic to animals. An inhalation carcinogenesis bioassay in mice and rats is to be conducted by NTP (Abdo, 1983). No unit risk estimate for HEX has been suggested because carcinogenic bioassay data for HEX have not been completed.

## 8.3. REGULATIONS AND STANDARDS

Hexachlorocyclopentadiene has been addressed under numerous U.S. statutes. These have been grouped according to the type of activity or medium being controlled.

**8.3.1. Occupational Standards.** There is no current OSHA standard for HEX levels in the workplace (29 CFR 1910). However, the ACGIH has adopted a threshold limit value (TLV), expressed as an 8-hour time-weighted average (TWA), of 0.1 mg/m$^3$ (0.01 ppm). A short-term exposure limit (STEL), the maximal concentration allowable in a 15-minute period, of 0.3 mg/m$^3$ (0.03 ppm) for HEX has also been adopted (ACGIH, 1982). The levels are based on the Treon et al. (1955) study.

In 1978, NIOSH classified HEX as a Group II pesticide and recommended criteria for standards for occupations in pesticide manufacturing and formulating. These standards rely on engineering controls, work practices and medical surveillance programs, rather than workplace air limits, to protect workers from the adverse effects of pesticide exposure in manufacturing and formulating. NIOSH specifically chose not to establish scientifically valid environmental (workplace air) limits for pesticides (except those already promulgated), because exposure by other routes, especially dermal, had proved to be of critical importance for many pesticides and because NIOSH believed that "immediate action" was needed to protect workers in pesticide manufacturing and formulating plants (NIOSH, 1978).

**8.3.2. Transportation Regulations.** The Hazardous Materials Transportation Act specifies the requirements to be observed in the preparation for shipment and transport of hazardous materials (49 CFR 171-179). The transport of HEX by air, land and water is regulated by these statutes, and the Department of Transportation has designated HEX as a "hazardous material" (ID Number UN 2646), a "corrosive material", and a "hazardous substance" (49 CFR 172.101). The maximum net quantity of HEX permitted in one package for transport by passenger-carrying aircraft or railcar has been set at 1 quart, while the maximum net quantity for cargo aircraft has been set at 10 gallons per package. Transport on deck or below deck by cargo vessel is also permitted (49 CFR 172.101).

The Hazardous Materials Transportation Act, in conjunction with the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), also provides that common carriers of hazardous substances may be held liable for releases of hazardous substances in amounts equal to or greater than their designated reportable quantity (RQ). The RQ for HEX has been set at 1 pound (0.454 kg) (49 CFR 172).

8.3.3. Solid Waste Regulations. Under the Resources Conservation and Recovery Act (RCRA), EPA has designated HEX as a hazardous toxic waste, Hazardous Waste No. U 130 (40 CFR 261.33), subject to disposal and permit regulations of Title 40, Code of Federal Regulations, Parts 262-265 and Parts 122-124. Hexachlorocyclopentadiene is a hazardous constituent of wastewater treatment sludge from the production of chlordane, wastewater and scrub water from the chlorination of cyclopentadiene in the production of chlordane, and filter solids from the filtration of HEX in the production of chlordane (Hazardous Waste Nos. K032, K033 and K034, respectively) which are also designated as a hazardous waste (40 CFR 261.320) and subject to RCRA disposal regulations.

**8.3.4. Food Tolerances.** Under FIFRA, a tolerance of 0.3 ppm has been established for technical chlordane, its components and metabolites which cannot contain >1% of HEX (40 CFR 180.122).

**8.3.5. Water Regulations.** Under section 311 of the Federal Water Pollution Control Act, HEX was designated as a hazardous substance (40 CFR 116.4) and these regulations established a Reportable Quantity (RQ) of 1 pound (0.454 kg) for HEX (40 CFR 117.3). Discharges equal to or greater than the RQ into or upon U.S. waters are prohibited unless the discharge is in compliance with applicable permit programs (40 CFR 117.11).

Under the Clean Water Act, EPA has designated HEX as a toxic pollutant; i.e., priority pollutant (40 CFR 401.15). Effluent limitations guidelines, new source performance standards, and pretreatment standards have been developed or will be developed for the priority pollutants for 21 major industries. Specific definitions for classes and categories are set forth in 40 CFR Parts 402 through 699.

Under the Clean Water Act, Ambient Water Quality Criteria (AWQC) for HEX have also been developed (U.S. EPA, 1980c). Based on available toxicity data for the protection of public health, the level derived was 206 $\mu g/\ell$. Using organoleptic data for controlling undesirable taste and odor quality of ambient water, the estimated level was 1 $\mu g/\ell$. The AWQC for freshwater aquatic life from acute and chronic toxicity indicated concentrations as low as 7.0 and 5.2 $\mu g/\ell$, respectively. Acute toxicity to saltwater aquatic life was indicated at concentrations as low as 7.0 $\mu g/\ell$ (U.S. EPA, 1980c).

**8.3.6. Air Regulations.** Hexachlorocyclopentadiene is not regulated under the Clean Air Act. The U.S. EPA will propose a decision on the need to regulate this chemical under the Clean Air Act and publish this proposal in the Federal Register.

**8.3.7. Other Regulations.** Pursuant to rules under sections 8(a) and 8(d) of the Toxic Substances Control Act (44 FR 31866), all manufacturers and processors of HEX are required to report health and safety information on HEX to EPA's Office of Toxic Substances. The deadline for submission of Preliminary Assessment Information Manufacturer's Report on HEX (40 CFR 712) was November 19, 1982.

In 1979, the Interagency Testing Committee recommended that HEX be considered for health and environmental effects testing under Section 4(a) of the TSCA (44 FR 31866). This recommendation was based on evidence of potential human exposure and a potential for environmental persistence and bioaccumulation. In 1982, the U.S. EPA responded (U.S. EPA, 1982) in the Federal Register. The following is an excerpt from that notice:

> EPA has decided not to initiate rulemaking to require testing of HEX under section 4 of TSCA because EPA does not believe that there is a sufficient basis to find that current manufacture, distribution in commerce, processing, use or disposal of HEX may present an unreasonable risk of injury to the environment or of mutagenic and teratogenic health effects. Neither has the EPA found evidence that there is substantial or significant environmental release of HEX. In addition, certain new studies have become available since the ITC's report or are underway, making additional testing for chronic and oncogenic effects unnecessary.

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# APPENDIX

## TABLE A-1

## Toxicity Table for Hexachlorocyclopentadiene

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit | gavage | 6 | | 420 mg/kg | 1 day | 1 exposure | NM | FEL | Peanut oil vehicle; exposure level 420-620 mg/kg, $LD_{50}$ Also toxic to heart, brain, kidney and liver | Treon et al., 1955 |
| Rat | gavage | 10 | | 280 mg/kg | 1 day | 1 exposure | NM | FEL | Male; peanut oil vehicle; minimum lethal dose 280 mg/kg | Treon et al., 1955 |
| Rat | gavage | 10 | | 280 mg/kg | 1 day | 1 exposure | NM | FEL | Female; peanut oil vehicle; minimum lethal dose 280 mg/kg | Treon et al., 1955 |
| Rat | gavage | NM | | 0 mg/kg | 1 day | 1 exposure | GI,LG | control | Females; all animals sacrificed 24 hours post-exposure | Komminent, 1978 |
| Rat | gavage | 10 | | 50 mg/kg | 1 day | 1 exposure | GI,LG | AEL | Exposure level range; 50-300 mg/kg | Komminent, 1978 |
| Rat | oral | 25 | | 530 mg/kg | 1 day | 1 exposure | NM | FEL | Female albinos; corn oil vehicle; $LD_{50}$ | IRDC, 1972 |
| Rat | oral | 25 | | 650 mg/kg | 1 day | 1 exposure | NM | FEL | Male albinos; corn oil vehicle; $LD_{50}$ | IRDC, 1972 |
| Rat | gavage | 10 | 0.107 | 75 mg/kg | 1 day | 1 exposure | GR,OT | NOFEL | Fischer 344 strain; both sexes, body weight range = (males: 101-133 g; females: 89-105 g); vehicle-corn oil | SRI, 1980a |
| Rat | gavage | 10 | 0.107 | 150 mg/kg | 1 day | 1 exposure | GR,OT | NOFEL | Fischer 344 strain; both sexes, body weight range = (males: 101-133 g; females: 89-105 g); vehicle-corn oil; animals in 75-150 mg/kg dose levels basically asymptomatic | SRI, 1980a |
| Rat | gavage | 10 | 0.107 | 300 mg/kg | 1 day | 1 exposure | GR,OT | FEL | 20% mortality; both females, on days 10 and 13, all effects more severe in females | SRI, 1980a |
| Rat | gavage | 10 | 0.107 | 600 mg/kg | 1 day | 1 exposure | GR,OT | FEL | 100% mortality; males by day 10 and females by day 6 | SRI, 1980a |
| Rat | gavage | 10 | 0.107 | 1200 mg/kg | 1 day | 1 exposure | GR,OT | FEL | 100% mortality by day 2 | SRI, 1980a |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Mouse | gavage | 10 | 0.021 | 75 mg/kg | 1 day | 1 exposure | GR,OT | NOFEL | B6C3F1 strain; both sexes; body weight range = (males: 20-24 g; females: 19-22 g); vehicle-corn oil; discoloration of urine noted | SRI, 1980a |
| Mouse | gavage | 10 | 0.021 | 150 mg/kg | 1 day | 1 exposure | GR,OT | NOFEL | B6C3F1 strain; both sexes; body weight range = (males: 20-24 g; females: 19-22 g); vehicle-corn oil; discoloration of urine noted; also noted ruffled fur but no change in activity | SRI, 1980a |
| Mouse | gavage | 10 | 0.021 | 300 mg/kg | 1 day | 1 exposure | GR,OT | NOFEL | B6C3F1 strain; both sexes; body weight range = (males: 20-24 g; females: 19-22 g); vehicle-corn oil; discoloration of urine noted; also noted ruffled fur but no change in activity; animals considered normal by day 6 | SRI, 1980a |
| Mouse | gavage | 10 | 0.021 | 600 mg/kg | 1 day | 1 exposure | GR,OT | FEL | 20% mortality-1 male and 1 female; effects same as 1200 mg/kg level but reversible by day 9 or 12 | SRI, 1980a |
| Mouse | gavage | 10 | 0.021 | 1200 mg/kg | 1 day | 1 exposure | GR,OT | FEL | 100% mortality by day 8; effects included decreased activity, ruffled fur and red urine | SRI, 1980a |
| Rat | gavage | 5 | 0.165 | 926 mg/kg | 1 day | 1 exposure | NM | FEL | Charles River CD strain; $LD_{50}$; vehicle-corn oil. Observed for 14 days post-exposure | IRDC, 1968 |
| Mouse | gavage | 10 |  | 0 mg/kg | 5 days |  | RP | control | CD-1 strain; males; DMSO administered as solvent vehicle | Litton Bionetics Inc., 1978b |
| Mouse | gavage | 10 |  | 0.1 mg/kg | 5 days |  | RP | NOFEL | Each male mated on day 7 to unexposed females. No evidence for significant dominant lethal activity | Litton Bionetics Inc., 1978b |
| Mouse | gavage | 10 |  | 0.3 mg/kg | 5 days |  | RP | NOFEL | Each male mated on day 7 to unexposed females. No evidence for significant dominant lethal activity; all values within control levels | Litton Bionetics Inc., 1978b |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Mouse | gavage | 10 | | 1 mg/kg | 5 days | | RP | NOFEL | Each male mated on day 7 to unexposed females. No evidence for significant dominant lethal activity; all values within control levels | Litton Bionetics Inc., 1978b |
| Rat | oral | 5 | | 0 mg/kg | 10 days | | RP | control | Charles River (CD); 12-week-old females; vehicle: corn oil; schedule: days 6-15 of gestation | IRDC, 1978 |
| Rat | oral | 5 | | 3 mg/kg | 10 days | | RP | NOEL | No teratogenic effects or change in maternal appearance or behavior | IRDC, 1978 |
| Rat | oral | 5 | | 10 mg/kg | 10 days | | RP | NOEL | No teratogenic effects or change in maternal appearance or behavior | IRDC, 1978 |
| Rat | oral | 5 | | 30 mg/kg | 10 days | | RP | EL | No teratogenic effects, decreased maternal body weight gain | IRDC, 1978 |
| Rat | oral | 5 | | 100 mg/kg | 10 days | | RP,GR | AEL | No teratogenic effects. Maternal body weight loss; reduced gain | IRDC, 1978 |
| Rat | oral | 5 | | 300 mg/kg | 10 days | | RP | FEL | 100% mortality by gestation day 10 | IRDC, 1978 |
| Mouse | gavage | NM | | 0 mg/kg | 10 days | | RP | control | CF-1; cottonseed oil vehicle; exposure schedule: days 6-15 of gestation | Murray et al., 1980 |
| Mouse | gavage | NM | | 5 mg/kg | 10 days | | RP | NOEL | No teratogenic, embryotoxic or fetotoxic effects | Murray et al., 1980 |
| Mouse | gavage | NM | | 25 mg/kg | 10 days | | RP | NOEL | No teratogenic, embryotoxic or fetotoxic effects; similar results in rabbit | Murray et al., 1980 |
| Mouse | gavage | NM | | 75 mg/kg | 10 days | | RP | NOEL | No teratogenic, embryotoxic or fetotoxic effects; similar results in rabbit | Murray et al., 1980 |
| Rat | gavage | 10 | 0.124 | 0 mg/kg | 12 days | | GR,GI | control | Fischer 344 strain, both sexes; body weight range = (males: 129-165 g; females: 74-128 g); vehicle-corn oil | SRI, 1980b |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Rat | gavage | 10 | 0.124 | 25 mg/kg | 12 days | | GR,GI | NOAEL | No deaths or significant gross or clinical effects; average weight gain slightly depressed in females; males were unaffected | SRI, 1980b |
| Rat | gavage | 10 | 0.124 | 50 mg/kg | 12 days | | GR,GI | AEL | No deaths or significant clinical effects; depression of average weight gain in both sexes; gross changes in stomach wall | SRI, 1980b |
| Rat | gavage | 10 | 0.124 | 100 mg/kg | 12 days | | GR,GI | AEL | No deaths; depression of average weight gain in both sexes; gross and clinical effects | SRI, 1980b |
| Rat | gavage | 10 | 0.124 | 200 mg/kg | 12 days | | GR,GI | FEL | Lethal to 1 (1/5) males, 4 (4/5) females; severe gross and clinical effects | SRI, 1980b |
| Rat | gavage | 10 | 0.124 | 400 mg/kg | 12 days | | GR,GI | FEL | Lethal to all males (5/5) and 4 (4/5) females; severe gross and clinical effects | SRI, 1980b |
| Mouse | gavage | 10 | 0.024 | 0 mg/kg | 12 days | | NS,GI | control | B6C3F1 strain; both sexes; body weight range = (males: 23-31 g; females: 19-22 g); vehicle-corn oil | SRI, 1980b |
| Mouse | gavage | 10 | 0.024 | 50 mg/kg | 12 days | | NS,GI | EL | No chemical related deaths; slight inactivity and stomach changes noted in both sexes (1/5 males and 1/5 females) | SRI, 1980b |
| Mouse | gavage | 10 | 0.024 | 100 mg/kg | 12 days | | NS,GI | AEL | No deaths in either sex; inactivity and stomach changes in all males (5/5) and 4 (4/5) females | SRI, 1980b |
| Mouse | gavage | 10 | 0.024 | 200 mg/kg | 12 days | | NS,GI | AEL | Lethal to 1 (1/5) males; all animals showed signs of clinical toxicity | SRI, 1980b |
| Mouse | gavage | 10 | 0.024 | 400 mg/kg | 12 days | | NS,GI | FEL | Lethal to 4 (4/5) males and all (5/5) females prior to day 7. Clinical and gross toxicity observed | SRI, 1980b |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Mouse | gavage | 10 | 0.024 | 800 mg/kg | 12 days |  | NS,GI | FEL | Lethal to all animals (10/10) prior to day 5; clinical toxicity but no gross observations | SRI, 1980b |
| Rat | gavage | 20 | 0.134 | 0 mg/kg | 13 weeks | 5 days/week | KD,GI | control | Fischer 344 strain; both sexes; vehicle: corn oil; body weight range = (males: 130-170 g; females: 99-135 g) | SRI, 1981a |
| Rat | gavage | 20 | 0.134 | 10 mg/kg | 13 weeks | 5 days/week | KD,GI | EL | White raised area on stomach in 1 (1/10) males | SRI, 1981a |
| Rat | gavage | 20 | 0.134 | 19 mg/kg | 13 weeks | 5 days/week | KD,GI | EL | Epithelial hyperplasia noted in 2 (2/10) females only. Appearance of other lesions also observed in vehicle controls and not necessarily chemically induced. | SRI, 1981a |
| Rat | gavage | 20 | 0.134 | 38 mg/kg | 13 weeks | 5 days/week | KD,GI | AEL | Increased severity of effects | SRI, 1981a |
| Rat | gavage | 20 | 0.134 | 75 mg/kg | 13 weeks | 5 days/week | KD,GI | FEL | Increased severity of effects; lethal to 1 (1/10) males and 1 (1/10) females | SRI, 1981a |
| Rat | gavage | 20 | 0.134 | 150 mg/kg | 13 weeks | 5 days/week | KD, GI | FEL | Lethal to 6 (6/10) males; depression of average weight gain in both sexes | SRI, 1981a |
| Mouse | gavage | 20 | 0.022 | 0 mg/kg | 13 weeks | 5 days/week | KD,GI | control | B6C3F1 strain; both sexes; vehicle: corn oil; body weight range = (males: 24-28 g; females: 17-20 g) | SRI, 1981b |
| Mouse | gavage | 20 | 0.022 | 19 mg/kg | 13 weeks |  | KD,GI | NOAEL | No significant pathological or clinical effects. Increased liver-to-body weight ratio. | SRI, 1981b |
| Mouse | gavage | 20 | 0.022 | 38 mg/kg | 13 weeks |  | KD,GI | EL | Mild epithelial hyperplasia and focal inflammation in 2 (2/10) males and 2 (2/9) females | SRI, 1981b |
| Mouse | gavage | 20 | 0.022 | 75 mg/kg | 13 weeks |  | KD,GI | AEL | Minimal toxic nephrosis in females; hyperplasia and inflammation of forestomach in both sexes | SRI, 1981b |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Mouse | gavage | 20 | 0.022 | 150 mg/kg | 13 weeks | 5 days/week | KD,GI | AEL | Increased severity of effects; depression in average weight gain in both sexes | SRI, 1981b |
| Mouse | gavage | 20 | 0.022 | 300 mg/kg | 13 weeks | 5 days/week | KD,GI | FEL | Lethal to all males and 3 (3/10) females. Toxic nephrosis noted in females only | SRI, 1981b |
| Rat | oral | 10 | | 20 mg/kg | 6 months | | NM | FEL | White rats; unspecified oral route; 20% mortality | Naishstein and Lisovskaya, 1965 |
| Rat | oral | 30 | 0.110 | 0.02 ug/kg | 6 months | | BL,GR | NOEL | Aqueous solution; unspecified oral route; total animal number = 90 Body weight range = 100-120 g | Naishstein and Lisovskaya, 1965 |
| Rat | oral | 30 | 0.110 | 0.2 ug/kg | 6 months | | BL,GR | NOEL | Aqueous solution; unspecified oral route; total animal number = 90 Body weight range = 100-120 g | Naishstein and Lisovskaya, 1965 |
| Rat | oral | 30 | 0.110 | 2 ug/kg | 6 months | | BL,GR | EL | Aqueous solution; unspecified oral route; total animal number = 90 Body weight range = 100-120 g | Naishstein and Lisovskaya, 1965 |
| Rat | inhalation | 4 | | 46.5 ppm | 30 minutes | | LG,NS | AEL | Exposure duration: 30-60 minutes, similar effects - rabbit, mouse, guinea pig; also toxic to growth, other organs | Treon et al., 1955 |
| Guinea pig | inhalation | 2 | | 7.2 ppm | 1 hour | | LG | NOFEL | Lethal to 0% of animals | Treon et al., 1955 |
| Guinea pig | inhalation | 2 | | 13.5 ppm | 1 hour | | LG | FEL | 50% mortality | Treon et al., 1955 |
| Guinea pig | inhalation | 2 | | 20 ppm | 1 hour | | LG | FEL | 100% mortality | Treon et al., 1955 |
| Rat | inhalation | 4 | | 3.1 ppm | 1 hour | | LG | NOFEL | 0% mortality | Treon et al., 1955 |
| Rat | inhalation | 4 | | 7.2 ppm | 1 hour | | LG | FEL | 50% mortality | Treon et al., 1955 |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Mouse | Inhalation | 5 |  | 1.4 ppm | 1 hour |  | LG | NOFEL | 0% mortality | Treon et al., 1955 |
| Mouse | Inhalation | 5 |  | 7.2 ppm | 1 hour |  | LG | FEL | 20% mortality | Treon et al., 1955 |
| Mouse | Inhalation | 5 |  | 13.8 ppm | 1 hour |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Rabbit | Inhalation | 3 |  | 1.4 ppm | 1 hour |  | LG | NOFEL | 0% mortality | Treon et al., 1955 |
| Rabbit | Inhalation | 3 |  | 3.1 ppm | 1 hour |  | LG | FEL | 67% mortality | Treon et al., 1955 |
| Rabbit | Inhalation | 3 |  | 7.2 ppm | 1 hour |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Rat | Inhalation | 4 |  | 20 ppm | 1.25 hour |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Guinea pig | Inhalation | 2 |  | 3.1 ppm | 3.5 hour |  | LG | NOFEL | Lethal to 0% of animals | Treon et al., 1955 |
| Guinea pig | Inhalation | 2 |  | 7.1 ppm | 3.5 hour |  | LG | FEL | 50% mortality | Treon et al., 1955 |
| Guinea pig | Inhalation | 2 |  | 12.4 ppm | 3.5 hour |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Rat | Inhalation | 4 |  | 1.4 ppm | 3.5 hour |  | LG | NOFEL | 0% mortality | Treon et al., 1955 |
| Rat | Inhalation | 4 |  | 3.1 ppm | 3.5 hour |  | LG | FEL | 50% mortality | Treon et al., 1955 |
| Rat | Inhalation | 4 |  | 7.1 ppm | 3.5 hour |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Mouse | Inhalation | 5 |  | 1.4 ppm | 3.5 hour |  | LG | FEL | 20% mortality | Treon et al., 1955 |
| Mouse | Inhalation | 5 |  | 3.1 ppm | 3.5 hour |  | LG | FEL | 80% mortality | Treon et al., 1955 |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Mouse | inhalation | 5 |  | 7.1 ppm | 3.5 hour |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Rabbit | inhalation | 3 |  | 6.4 ppm | 3.5 hour |  | LG | FEL | 67% mortality | Treon et al., 1955 |
| Rabbit | inhalation | 3 |  | 7.1 ppm | 3.5 hour |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Rat | inhalation | 10 |  | 176.2 ppm | 4 hours |  | LG,SK | FEL | 100% mortality within 48 hours; also toxic/other organs | IRDC, 1972 |
| Rat | inhalation | 10 |  | 17,624 ppm | 4 hours |  | LG,SK | FEL | 100% mortality within the 48-hour exposure period | IRDC, 1972 |
| Rat | inhalation | 10 | 0.250 | 1.6 ppm | 4 hours |  | LG,GR | FEL | Sprague-Dawley strain; males (200-300 g) $LC_{50}$ | Rand et al., 1982 |
| Rat | inhalation | 10 | 0.250 | 3.5 ppm | 4 hours |  | LG,GR | FEL | Sprague-Dawley strain; females (200-300 g) $LC_{50}$ | Rand et al., 1982 |
| Guinea pig | inhalation | 2 |  | 1.5 ppm | 7 hours |  | LG | NOFEL | Lethal to 0% of animals | Treon et al., 1955 |
| Guinea pig | inhalation | 2 |  | 3.2 ppm | 7 hours |  | LG | FEL | 50% mortality | Treon et al., 1955 |
| Guinea pig | inhalation | 2 |  | 6.7 ppm | 7 hours |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Rat | inhalation | 4 |  | 1.5 ppm | 7 hours |  | LG | FEL | 25% mortality | Treon et al., 1955 |
| Rat | inhalation | 4 |  | 3.2 ppm | 7 hours |  | LG | FEL | 75% mortality | Treon et al., 1955 |
| Rat | inhalation | 4 |  | 6.7 ppm | 7 hours |  | LG | FEL | 100% mortality | Treon et al., 1955 |
| Mouse | inhalation | 5 |  | 1.5 ppm | 7 hours |  | LG | FEL | 80% mortality | Treon et al., 1955 |
| Mouse | inhalation | 5 |  | 3.2 ppm | 7 hours |  | LG | FEL | 100% mortality | Treon et al., 1955 |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit | inhalation | 3 | | 7.5 ppm | 7 hours | | LG | FEL | 100% mortality | Treon et al., 1955 |
| Rat | inhalation | 20 | 0.162 | 0 ppm | 14 days | 6 hours/day<br>5 days/week | LG,BL | control | Range finding study, Sprague-Dawley strain; both sexes (136-188 g) | Rand et al., 1982 |
| Rat | inhalation | 20 | 0.162 | 0.022 ppm | 14 days | 6 hours/day<br>5 days/week | LG,BL | NOAEL | No significant clinical or pathological effects | Rand et al., 1982 |
| Rat | inhalation | 20 | 0.162 | 0.11 ppm | 14 days | 6 hours/day<br>5 days/week | LG,BL | EL | Decreased body weight with increased liver weight; males more affected than females | Rand et al., 1982 |
| Rat | inhalation | 20 | 0.162 | 0.5 ppm | 14 days | 6 hours/day<br>5 days/week | LG,BL | AEL | Pathological and blood chemistry changes. Also toxic to liver, kidney, nasal passage. Dose-related effects | Rand et al., 1982 |
| Rabbit | inhalation | 6 | | 0.34 ppm | 35 days | 5 days/week | GR | FEL | Lethal to 4 (4/6) animals | Treon et al., 1955 |
| Guinea pig | inhalation | 2 | | 0.34 ppm | 42 days | 7 hours/day<br>5 days/week | GR | AEL | Guinea pigs survived 30 periods of exposure; lethal to mice and rats similarly exposed before 20th exposure period | Treon et al., 1955 |
| Rat | inhalation | 80 | 0.192 | 0 ppm | 90 days | 6 hours/day<br>5 days/week | LG,BL | control | Sprague-Dawley strain; both sexes (160-224 g) | Rand et al., 1982 |
| Rat | inhalation | 80 | 0.192 | 0.01 ppm | 90 days | 6 hours/day<br>5 days/week | LG,BL | NOAEL | No measurable clinical or physical effects | Rand et al., 1982 |
| Rat | inhalation | 80 | 0.192 | 0.05 ppm | 90 days | 6 hours/day<br>5 days/week | LG,BL | EL | Marginal hematologic and organ weight changes | Rand et al., 1982 |
| Rat | inhalation | 80 | 0.192 | 0.2 ppm | 90 days | 6 hours/day<br>5 days/week | LG,BL | EL | Marginal hematologic and organ weight changes | Rand et al., 1982 |
| Monkey | inhalation | 12 | 2.000 | 0 ppm | 90 days | 6 hours/day<br>5 days/week | LG,BL | control | Cynomolgus monkeys; both sexes (1.5-2.5 kg) | Rand et al., 1982 |
| Monkey | inhalation | 12 | 2.000 | 0.01 ppm | 90 days | 6 hours/day<br>5 days/week | LG,BL | NOAEL | No treatment-related abnormalities - organ weights, pathology or histopathology | Rand et al., 1982 |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Monkey | inhalation | 12 | 2.000 | 0.05 ppm | 90 days | 6 hours/day<br>5 days/week | LG,BL | NOAEL | No treatment-related abnormalities - organ weights, pathology or histopathology | Rand et al., 1982 |
| Monkey | inhalation | 12 | 2.000 | 0.2 ppm | 90 days | 6 hours/day<br>5 days/week | LG,BL | NOAEL | No treatment-related abnormalities - organ weights, pathology or histopathology | Rand et al., 1982 |
| Guinea pig | inhalation | 2 | | 0.15 ppm | 216 days | 7 hours/day<br>5 days/week | GR,LV, KD | AEL | Concentration tolerated by guinea pigs, rabbits and rats; lethal to 4 (4/5) mice similarly exposed. Mild degenerative changes noted in LV and KD | Treon et al., 1955 |
| Rat | inhalation | 4 | | 12.4 ppm | NM | | LG,NS | AEL | Exposure level: 12.4-13.8 ppm, similar effects in rabbit, mouse, guinea pig | Treon et al., 1955 |
| Rat | inhalation | 4 | | 1 ppm | NM | | LG,NS | AEL | Exposure level: 1-1.6 ppm; symptoms developed over a period of hours | Treon et al., 1955 |
| Rat | inhalation | 4 | | 0.15 ppm | NM | | LG,NS | EL | Exposure level: 0.15-0.33 ppm; irritation seen only in mouse | Treon et al., 1955 |
| Human | inhalation | 145 | | 0.4 mg/ℓ | NM | | NS,MT | AEL | Epidemiological study; exposure to mixture of HEX and octachlorocyclopentene. Exposure level: 100-1000 ppm in wastewater | Singal, 1978 |
| Rabbit | dermal | 6 | | 430 mg/kg | 1 day | 1 exposure | NM | FEL | Painted; lethal dosage range: 430-630 mg/kg | Treon et al., 1955 |
| Guinea pig | dermal | 1 | | 0 mg/kg | 1 day | 1 exposure | LG,SK | control | Painted; animals sacrificed 24 hours post-exposure | Komminen1, 1978 |
| Guinea pig | dermal | 1 | | 300 mg/kg | 1 day | 1 exposure | LG,SK | AEL | Painted; animals sacrificed 24 hours post-exposure | Komminen1, 1978 |
| Guinea pig | dermal | 1 | | 600 mg/kg | 1 day | 1 exposure | LG,SK | AEL | Painted; animals sacrificed 24 hours post-exposure | Komminen1, 1978 |
| Guinea pig | dermal | 1 | | 1200 mg/kg | 1 day | 1 exposure | LG,SK | FEL | Expired prior to sacrifice | Komminen1, 1978 |

TABLE A-1 (cont.)

| Species | Route | Number of Animals | Body Weight | Exposure Level | Duration of Exposure | Exposure Schedule | Organ | Severity | Comments | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit | dermal | 4 | | 200 mg/kg | 1 day | 1 exposure | GR,SK | FEL | Painted; New Zealand white, both sexes; lethal/both males | IRDC, 1972 |
| Rabbit | dermal | 4 | | 2000 mg/kg | 1 day | 1 exposure | GR,SK | FEL | Painted; New Zealand white, both sexes; lethal/both males; mortality within 24 hours | IRDC, 1972 |
| Rabbit | dermal | 3 | | 250 mg/kg | 1 day | 1 exposure | SK | AEL | Dose-related effects persisting for many days | Treon et al., 1955 |
| Monkey | dermal | 1 | | NM | 3 days | | SK | AEL | Exposure level = 0.05 mℓ of 10% ultrasene solution. Increased solution concentration (20, 40, 60, 90%) produced more severe effects | Treon et al., 1955 |
| Rabbit | dermal | NR | | NM | 10 days | | SK | NOEL | Exposure level = 0.5-0.6 mℓ of 20 ppm HEX in aqueous solution | Naishstein and Lisovskaya, 1965 |
| Monkey | dermal | 1 | | NM | NM | | SK | NOEL | Exposure level = 0.01 mℓ of 0.001-10% ultrasene solution; similar effect in guinea pig | Treon et al., 1955 |

NM = Not mentioned

BL = Blood; GI = gastrointestinal; GR = growth/weight gain; KD = kidney; LG = lung; MT = metabolism; NS = nervous system including CNS; OT = other; RP = reproductive; SK = skin

NOEL = No observed effect level. That exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and the appropriate control.

NOAEL = No observed adverse effect level. That exposure level at which there are no statistically significant increases in frequency or severity of adverse effects between the exposed population and the appropriate control. Effects are produced at this level, but they are not considered to be adverse.

EL = Effect level. The exposure level in a study or group of studies which produces statistically significant increases in frequency or intensity of effects between the exposed population and its appropriate control. It has not been decided whether these effects are adverse.

AEL = Adverse effect level. The exposure level in a study or group of studies which produces statistically significant increases in frequency or severity of adverse effects between the exposed population and the appropriate control.

NOFEL = No observed frank effect level. The study was directed toward eliciting frank effects, but none were observed of statistical significance. Other less severe toxic effects may have been present but were not investigated.

FEL = Frank effect level. That exposure level which produces unmistakable adverse effects or gross toxicity, such as irreversible functional impairment or mortality, at a statistically significant increase in frequency or severity between an exposed population and its appropriate control.

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